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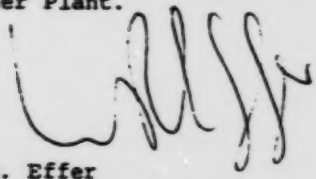
Subject Effects of Temperature and pH on the
Short-Term Toxicity of Hydrogen Sulphide
to Sac-Fry and Juvenile Great Lakes Fish

The Ontario Hydro Bruce Heavy Water Plant uses hydrogen sulphide in the preparation of heavy water for use as a moderator in CANDU nuclear power plants. The process water discharged back into the water body contains traces of hydrogen sulphide which remain after the stripping process.

Hydrogen sulphide is known to be highly toxic to fish, especially in the aqueous, undissociated form. In order to negotiate new discharge guidelines with the Ministry of the Environment, Ontario Hydro has carried out a number of related studies. They include dissociation versus toxicity¹, emission limits², and toxicity to Lake Huron fish species³.

The attached report by Dr. J.S. Griffiths describes recent studies which allow prediction of H₂S toxicity levels over the seasonal range of pH and temperature that exists in the Heavy Water Plant discharge area.

Results suggest general agreement with previous and other studies, i.e., toxicity of H₂S increases with temperature and decreases with rise in pH, reflecting the toxic effect of the undissociated species. However, these results also give further evidence of the toxic nature, albeit slight, of the HS⁻ ion species⁴. In some cases, the results show substantially greater resistance to H₂S than previously reported by Fung and Bewick³, and toxic levels are generally well above the maximum allowable emission limits for the Bruce Heavy Water Plant.



W.R. Effer
Manager
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D.K. Fung/vc

circ: C.D. Burnham/D.K. Fung - H10

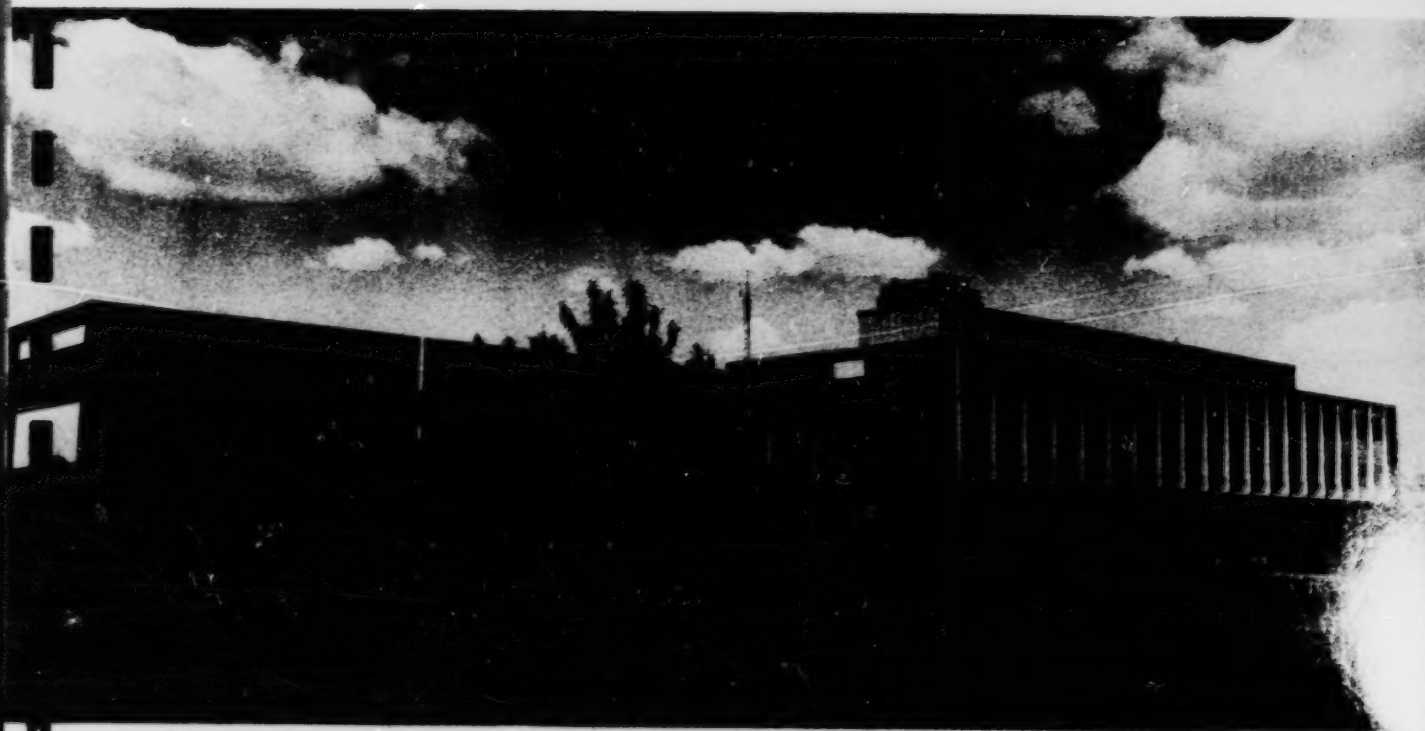
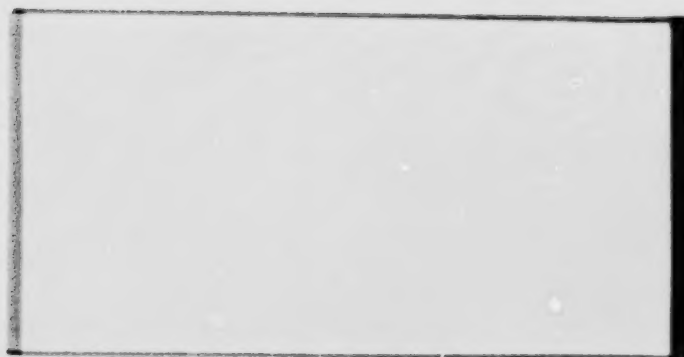
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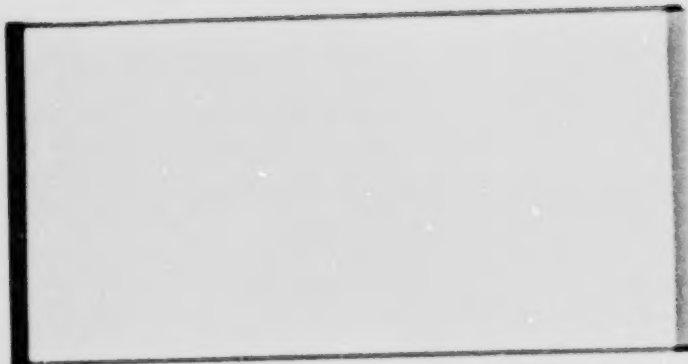
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2. Guest, A., 1978, "Environmental Effects of H₂S Emissions to Lake Huron from Bruce Heavy Water Plant", Ontario Hydro Environmental Protection Section Report No. RMEP-IR-090-9.
3. Fung, D.K. and P.H. Bewick, "Short-Term Toxicity of Aqueous Hydrogen Sulfide to Representative Fish Species of Lake Huron", Aquatic Toxicology, ASTM STP 707, J.G. Eaton, P.R. Parrish and A.C. Hendricks, Eds., American Society for Testing Materials, 1980, pp. 377-396.
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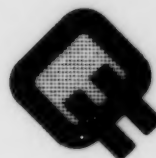
Named in honour of Dr. W.P. Dobson, the Research Division's director from 1914 to 1953, the building shown on the front cover was first occupied in 1961. Along with the adjacent pump testing complex and the high-voltage, high-current and radioactive materials laboratories, it accommodates much of the research, development and testing work performed in support of the diverse technical needs of Ontario Hydro.

The Research Division's staff is engaged on a wide range of problems relating to the design, construction, operation and maintenance of the power system and to the efficient, economical use of electric power.

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**EFFECTS OF TEMPERATURE AND pH ON THE
SHORT-TERM TOXICITY OF HYDROGEN
SULPHIDE TO SAC-FRY AND JUVENILE
GREAT LAKES FISH**

Report No 83-121-K

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ABSTRACT

Toxicity of H_2S to sac-fry and juveniles of several Great Lakes fish species was determined in 1 hour and 96 hour bioassays, considering the seasonal range of temperature and pH expected in the Bruce Heavy Water Plant discharge to Lake Huron. Response surface models fitted to results from factorial experiments defined relations between mortality, H_2S levels and temperature at pH levels of 7.5, 8.0 and 8.5. Under most conditions, the following order of sensitivity to H_2S was observed: round whitefish sac-fry > lake whitefish sac-fry > rainbow trout sac-fry > rainbow trout juveniles > rock bass sac-fry. The toxicity of H_2S increased with temperature in a manner suggesting a link between uptake of H_2S and gill ventilation rates. Toxicity of a given level of total H_2S declined as pH increased, as less of the most toxic (aqueous) form was present at the higher pH. However, as sensitivity to a given level of aqueous H_2S increased over the pH range of 7.5 to 8.5, a slight toxicity of the HS^- ion (abundant at high pH) was suggested. Present results in most cases indicated substantially greater resistance to H_2S than reported by MacLaren (1977) under comparable conditions. Toxic H_2S levels determined in the present study were generally well above the maximum emission limits for the Bruce Heavy Water Plants.

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ontario hydro research division

To Dr. W.R. Effer
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EFFECTS OF TEMPERATURE AND pH ON THE SHORT-TERM TOXICITY OF HYDROGEN SULPHIDE TO SAC-FRY AND JUVENILE GREAT LAKES FISH

INTRODUCTION

Ontario Hydro's CANDU nuclear reactors use heavy water (deuterium oxide, D₂O) as a moderator and primary heat transfer medium within the reactor core. This heavy water is obtained from natural lake water in extraction plants located at the Bruce Nuclear Power Development on Lake Huron. The Bruce Heavy Water Plants use H₂S as an extraction medium. In this process, D₂O in lake water (warmed to 27°C) is dissolved in H₂S, resulting in a solution of about 13% D₂O (compared with about 148 ppm in natural water). The solution is then fed to a series of flash drums and an effluent stripper, where most of the H₂S is removed and recycled for use with incoming process water. The heavy water solution is then vacuum distilled to upgrade the D₂O content to 99.75%.

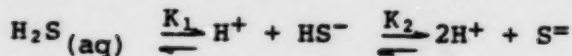
The used process water is mixed with the plant cooling water, resulting in a maximum discharge ΔT of 11.1°C. Small amounts of H₂S remain in the discharge water. Generally, concentrations are well below 50 ppb. However, process upsets in the enriching units may occasionally lead to higher concentrations in process water effluent streams. Hence, interest exists in establishing the toxicity of this effluent stream to Lake Huron fish species over both longer term conditions and during the transient, very short term conditions of process upsets. A. Guest (Ontario Hydro RMEP Report IR-090-9) reviewed the pertinent literature and recommended to the Ministry of the Environment the following emission limits (expressed as total sulphide):

175 ppb averaged over 1 hour
95 ppb averaged over 24 hours
20 ppb averaged over 1 year

After some discussion, the Ministry of the Environment concurred with A. Guest's findings. However, to ensure an even larger safety margin, further reduced these emission limits to the following (expressed as total sulphide):

105 ppb averaged over 1 hour
82 ppb averaged over 24 hours

In water, hydrogen sulphide dissociates as follows:



where K₁ and K₂ are the first and second acid dissociation

constants of hydrogen sulphide. As the concentration of $S^{=}$ is negligible at a pH below 10, the second dissociation constant may be ignored for Lake Huron waters (Clarke, 1974, Ontario Hydro CNS Report IR-191). Lake Huron pH levels normally range from pH 7.8 to pH 8.3, with extreme seasonal values occasionally approaching the pH 7.5 to pH 8.5 range. The dissociation of $H_2S(aq)$ to H^+ and HS^- is highly pH dependent, is also altered by temperature, and to a lesser degree, by ionic strength. Clarke (1974) indicated that at pH 7.5 and $10^\circ C$, about 33% of total H_2S dissolved in Lake Huron water would be in the undissociated (aqueous) form, while at pH 8.5 and $10^\circ C$, only 4.7% would be in the aqueous form. At pH 7.5 and $20^\circ C$, about 27% of the total sulphide would be undissociated. As aqueous H_2S is assumed to be the toxic form of hydrogen sulphide (Clarke, 1974; Fung and Bewick, 1978), it is clear that a heated H_2S discharge to Lake Huron could vary considerably in toxicity due to the seasonal temperature and pH progression.

The majority of H_2S toxicity studies reported in the literature have been aimed at determining effects of H_2S produced in organically enriched sediments, ie, areas affected by pulp mill or domestic wastes. Hence such studies have been conducted under conditions of low oxygen and lower pH than that common to the Bruce Heavy Water Plant site. Ontario Hydro commissioned a study of H_2S toxicity to Lake Huron fish species by J.F. MacLaren (1977). In that study, sensitive life stages (primarily sac-fry and juveniles) of four species were tested under temperature/pH combinations representing summer and winter conditions at the BHWP site. The winter condition was taken as pH 7.5 and $7^\circ C$, while the summer condition was taken as pH 8.0 and the final preferendum temperature of the species concerned, ranging from $10^\circ C$ for lake whitefish to $25^\circ C$ for largemouth bass. Both 1 hour and 96 hour bioassay tests were run. Results indicated that H_2S toxicity to fish depended upon the species and life stage tested, with sac-fry being generally the least resistant stage (MacLaren, 1977; Fung and Bewick, 1978). However, because of experimental design limitations, it was not possible to determine the independent effects of pH and temperature on H_2S toxicity.

Clarke (1974), MacLaren (1977) and Fung and Bewick (1978) have assumed that H_2S toxicity was relatively independent of pH when $H_2S(aq)$ values were calculated, ie, that aqueous H_2S was the only toxic species of a hydrogen sulphide solution. However, Guest (1978, Ontario Hydro RMEP Report IR-090-9) presented data from Smith et al (1976) which indicated a substantial increase in the toxicity of $H_2S(aq)$ with increasing pH. The data suggested a fourfold increase in sensitivity to H_2S over the pH 6.5 to pH 8.5 range for the test species (fathead minnow). However, it should be realized that to maintain a given aqueous H_2S level over this pH range, the amount of total H_2S present would increase manyfold, as a very large percentage of the total sulphide present would be in the form of HS^- at the higher pH.

To obtain a level of 50 ppb $\text{H}_2\text{S}_{(\text{aq})}$ at pH 6.5 and 15°C, 61.8 ppb total H_2S would be required (Clarke, 1974). At pH 8.5, a concentration of 1228 ppb total H_2S would be required to maintain the 50 ppb $\text{H}_2\text{S}_{(\text{aq})}$ level. Hence, if the HS^- ion has even a minor toxic effect, the huge increase in its concentration at high pH could account for the data presented by Smith et al (1976).

As some studies (Bonn and Follis, 1967; limited comparisons from MacLaren, 1977) do not indicate an increased sensitivity to $\text{H}_2\text{S}_{(\text{aq})}$ with increased pH, Guest (1978) concluded that further studies were necessary to define effects of pH on fish response to aqueous H_2S .

As previously indicated, temperature affects the dissociation of H_2S , resulting in proportionally less of the aqueous form at high temperatures. Results presented by Smith et al (1976) for fathead minnow and goldfish indicate a linear decline in the 96 hour LC50 for $\text{H}_2\text{S}_{(\text{aq})}$ with increased temperature when the data are plotted on logarithmic scales. However, these authors indicated that the relationship between the $\text{H}_2\text{S}_{(\text{aq})}$ LC50 and temperature for the brook trout was non-linear, declining from 8°C to 13°C, then remaining relatively constant or rising slightly at higher temperatures. Thus, it appears that additional data are required to determine responses of various species to temperature and hydrogen sulphide.

The Biological Research Section was contracted by the Environmental Studies and Assessments Department of Ontario Hydro to undertake further studies of H_2S toxicity. These studies were designed to verify the earlier MacLaren work, using comparable species/life stages, but experiments were run in triplicate, as the MacLaren studies were not replicated. The second objective of the present study was to expand the base of information, allowing prediction of H_2S toxicity levels over the seasonal range of pH and temperature expected for the Bruce Heavy Water Plant discharge. This was done by systematically augmenting the MacLaren experimental design to form modified factorial designs in three factors (H_2S level, pH, temperature). Both 1 hour and 96 hour bioassays were conducted on sensitive life stages (primarily sac-fry) of four Great Lakes species: rainbow trout, lake whitefish, round whitefish, and rock bass.

Results were analyzed by response surface methods outlined by Lindsey et al (1970) to provide predictive models. From these models, effects of a heated H_2S discharge could be estimated at any season. In addition, the separate and combined effects of temperature and pH on H_2S toxicity would be clearly evident.

METHODS

1. Experimental Fish

Sac-fry of four Great Lakes fish species (lake whitefish, round whitefish, rainbow trout, rock bass) were tested in 1 hour and 96 hour bioassays. Rainbow trout juveniles were also tested. In addition, a series of one hour experiments were run with rainbow trout fry (approximately 2 months old) to establish test procedures. In these latter experiments (unlike the other tests) the experimental fish were not acclimated to test temperature and pH levels prior to testing.

Lake whitefish (*Coregonus clupeaformis*) eggs were obtained from the Ministry of Natural Resources staff at Maple. The eggs were collected from fish spawning in Lake Simcoe, on November 21, 1978. Shortly after fertilization, they were transferred to the Dobson Research Laboratory where they were incubated through the winter of 1978-79 at an average temperature of 3.56°C (range 2.2 to 6.7°C). Testing of sac-fry began in the spring of 1979. Sac-fry of each species were acclimated to test temperatures and pH for 3 days prior to testing.

Round whitefish (*Prosopium cylindraceum*) eggs were collected by Ontario Hydro Biological Research field crews from Lake Ontario near Darlington GS on December 11, 1978. After fertilization in the field, eggs were incubated at the Dobson Research Laboratory over the winter of 1978-79 at an average temperature of 4.4°C (range 3.3 to 5.7°C). Bioassay tests on sac-fry were begun in the spring of 1979. Where possible, round whitefish and lake whitefish sac-fry were tested concurrently to reduce experimental costs.

Rainbow trout (*Salmo gairdneri*) were obtained as eggs (for sac-fry tests) or fry (for juvenile tests) from Shamrock Springs Trout Hatchery in Erin, Ontario. Two groups of sac-fry were tested. The first group was obtained as freshly fertilized eggs on November 29, 1978 and were incubated at the Dobson Laboratory at 3°C (range 1.0 to 7.5°C). These were used for 1 hour bioassays in the spring of 1979. The second group of eggs was fertilized on November 22, 1979, and incubated at 6.7°C (range 4.2 to 8.7°C). These were tested as sac-fry in 1 hour and 96 hour bioassays in early 1980. Rainbow trout fry (approximately 4 cm long) were obtained from Shamrock Springs on November 26, 1978. Some of these fish were used for initial "fry" tests in the early spring of 1979. The remainder were reared at the Dobson Research Laboratory until their use in "juvenile" (15 cm in length) 1 hour and 96 hour bioassays during the winter of 1979-80.

Rock bass (*Ambloplites rupestris*) were collected as newly fertilized eggs by Biological Research field crews from nests in

Baie du Dore, Lake Huron. The eggs were incubated at the Dobson Research Laboratory. Mean incubation temperatures for 1 hour sac-fry tests at pH 7.5 were 27.2°C and 15.6°C (two tests required to define toxicity levels). Fish tested at pH 8.0 were incubated at 22.3°C, while those tested at pH 8.5 were incubated at 10.6°C. This lower incubation temperature was used to delay hatch in order that the test series might be completed with newly hatched fry. As the egg developmental period and the yolk-sac stage of rock bass are very brief, it was not possible to conduct both 1 hour and 96 hour bioassay tests during 1979. Due to time and financial constraints, it was decided (after consultation with D.K. Fung, Environmental Studies and Assessments) not to attempt completion of the 96 hour tests the following year. Hence, only 1 hour bioassay tests were completed with rock bass sac-fry.

The original study proposal included tests with yellow perch sac-fry (August 28, 1978 memo to A.W. Rogers). Due to repeated tests required to establish toxic H₂S levels for rainbow trout and lake whitefish (MacLaren's 1977 results appeared to be in error) little time was available in the spring of 1979 for these tests. However, some perch eggs were obtained from fish held at the Dobson Laboratory, and these eggs were incubated to produce a stock of sac-fry. It is suspected that these eggs had been retained by the fish beyond the normal spawning time, as egg mortality was unusually high. Hatched fry showed excessive mortality, preventing the bioassay tests from being run. After consultation with D.K. Fung of Environmental Studies and Assessments, yellow perch were deleted from the testing schedule. Test conditions for each species/life stage are summarized in Table 1.

2. Control of Experimental Conditions

All tests were conducted as flow-through bioassays using dechlorinated Toronto tap water (from Lake Ontario). During the bioassay experiments, water quality samples were analyzed by the Analytic Services Section of Ontario Hydro. These results are presented in Appendix 1. A description of the bioassay apparatus and its operation follows.

A. pH Control and Monitoring

Due to the substantial effects of pH on the dissociation of H₂S, dependable and consistent pH control is a prerequisite for H₂S bioassay studies. A pH control and monitoring system was constructed which was capable of controlling experimental pH for the entire bioassay flow (approximately 11.5 liters per minute).

As temperature was controlled primarily by mixing chilled and heated supplies, it was necessary to separately control the pH of these two supplies prior to delivery to the test tanks. Two 250 liter polyethylene mixing containers were used for heated and chilled water, respectively. Flow into these containers from the general laboratory water system was controlled by float valves.

TABLE 1

TEST CONDITIONS FOR H_2S BIOASSAYS

Test Duration (hours)	Species	Life Stage	Temperatures (°C)	pH	Time of Testing
1	Rainbow Trout	Sac Fry	4, 8, 14, 20, 24	7.5	Group 1 Spring 1979
1	Rainbow Trout	Sac Fry	4, 8, 13, 18, 22	7.5	
1	Rainbow Trout	Sac Fry	4, 8, 13, 18, 22	8.0	
1	Rainbow Trout	Sac Fry	4, 8, 13, 18, 22	8.5	
1	Rainbow Trout	Sac Fry	4, 8, 12, 16, 20	7.5	Group 2 Winter-Spring 1980
1	Rainbow Trout	Sac Fry	4, 8, 12, 16, 20	8.0	
1	Rainbow Trout	Sac Fry	4, 8, 12, 16, 20	8.5	
1	Rainbow Trout	Fry	4, 8, 14, 20, 24	7.5	Early Spring 1979
1	Rainbow Trout	Fry	4, 8, 14, 20, 24	8.0	
1	Rainbow Trout	Fry	4, 8, 14, 20, 24	8.5	
1	Rainbow Trout	Juvenile	4, 8, 12, 16, 20	7.5	Winter 1979-80
1	Rainbow Trout	Juvenile	4, 8, 12, 16, 20	8.0	
1	Rainbow Trout	Juvenile	4, 8, 12, 16, 20	8.5	
1	Lake Whitefish	Sac Fry	4, 7, 10, 13, 16	7.5	Spring 1979
1	Lake Whitefish	Sac Fry	4, 7, 10, 13, 16	8.0	
1	Lake Whitefish	Sac Fry	4, 7, 10, 13, 16	8.5	
1	Round Whitefish	Sac Fry	4, 7, 10, 13, 16	7.5	Spring 1979
1	Round Whitefish	Sac Fry	4, 7, 10, 13, 16	8.0	
1	Round Whitefish	Sac Fry	4, 7, 10, 13, 16	8.5	
1	Rock Bass	Sac Fry	12, 16, 20, 24, 28	7.5	Summer 1979
1	Rock Bass	Sac Fry	10, 14, 18, 22, 26	7.5	
1	Rock Bass	Sac Fry	12, 16, 20, 24, 28	8.0	
1	Rock Bass	Sac Fry	12, 16, 20, 24, 28	8.5	
96	Rainbow Trout	Sac Fry	4, 8, 12, 16, 20	7.5	Winter 1979-80
96	Rainbow Trout	Sac Fry	4, 8, 12, 16, 20	8.0	
96	Rainbow Trout	Sac Fry	4, 8, 12, 16, 20	8.5	
96	Rainbow Trout	Juvenile	4, 8, 12, 16, 20	7.5	Winter 1979-80
96	Rainbow Trout	Juvenile	4, 8, 12, 16, 20	8.0	
96	Rainbow Trout	Juvenile	4, 8, 12, 16, 20	8.5	
96	Lake Whitefish	Sac Fry	4, 7, 10, 13, 16	7.5(3)	Spring 1979
96	Lake Whitefish	Sac Fry	4, 7, 10, 13, 16	8.0	
96	Lake Whitefish	Sac Fry	4, 7, 10, 13, 16	8.5	
96	Round Whitefish	Sac Fry	4, 7, 10, 13, 16	7.5(3)	Spring 1979
96	Round Whitefish	Sac Fry	4, 7, 10, 13, 16	8.0	
96	Round Whitefish	Sac Fry	4, 7, 10, 13, 16	8.5	

The pH in each container was monitored by a Corning model 125 digital pH meter. Outputs were recorded on a dual channel Hewlett-Packard model 7100 BM strip chart recorder, providing a continuous and permanent pH record for each bioassay test.

As the total flow through the bioassay system was relatively constant (dilution flows were metered) it was initially assumed that adequate pH control could be obtained by manually adding (gravity flow) base (NaOH) or acid (HCl) solution to raise or lower pH to the level desired for a particular test. However, daily variations in the pH of incoming water prevented this approach from being adequate. Therefore, the pH monitoring system was modified to become a controlling system. Micro-switches were fitted to the recorder pens. When pH in the mixing tank varied from the desired value, the micro-switches activated a solenoid. This solenoid controlled a pinch clamp, which when released, allowed the desired acid or base to flow into the mixing tank. When pH returned to the desired value, the micro-switch was again triggered, shutting off the acid/base inflow. The system is shown in Figure 1. This system provided quite precise pH control in the mixing tanks (generally within ± 0.05 pH units). Following mixing of the heated and chilled supplies, and aeration in header tanks before delivery to test tanks, some variation in pH levels occurred. However, confidence intervals of recorded values (in test tanks) over the 96 hour bioassays were generally better than ± 0.1 pH units.

B. Temperature Control

Laboratory supplies of dechlorinated heated and chilled water were fed into the two 250 liter mixing tanks where pH adjustment occurred. A portable chiller (Ranco Frigid Units) provided additional cooling capacity to maintain the chilled supply below 4°C . Water from the heated and chilled mixing tanks was pumped to four mixing valves (Powers Model 1 Fotopanel) which provided the desired test temperatures. Four constant-head, 50 liter delivery tanks equipped with float valves were supplied directly from the mixing valves. Water in a fifth head tank, which provided the warmest test temperature, was further heated by a 1000 watt immersion heater controlled by a YSI Model 72 proportional temperature controller. Water in all head tanks was vigorously aerated to assure oxygen levels near saturation in the dilution water. The dilution water was delivered to the individual test tanks by gravity flow, as the head tanks were located about 2 meters above the test tanks.

C. Dissolved H_2S Generator

A dissolved hydrogen sulphide generator was constructed to supply a concentrated H_2S solution for mixing with the dilution water. This apparatus (Figure 2) consisted of a 12 cm diameter clear PVC tube 120 cm in length, closed at each end with PVC plumbing caps. Water flow through the H_2S generator was controlled by a calibrated PVC needle valve and a flowmeter (Roger Gilmont Size 5). Inflow water and H_2S gas were introduced 5 cm from the

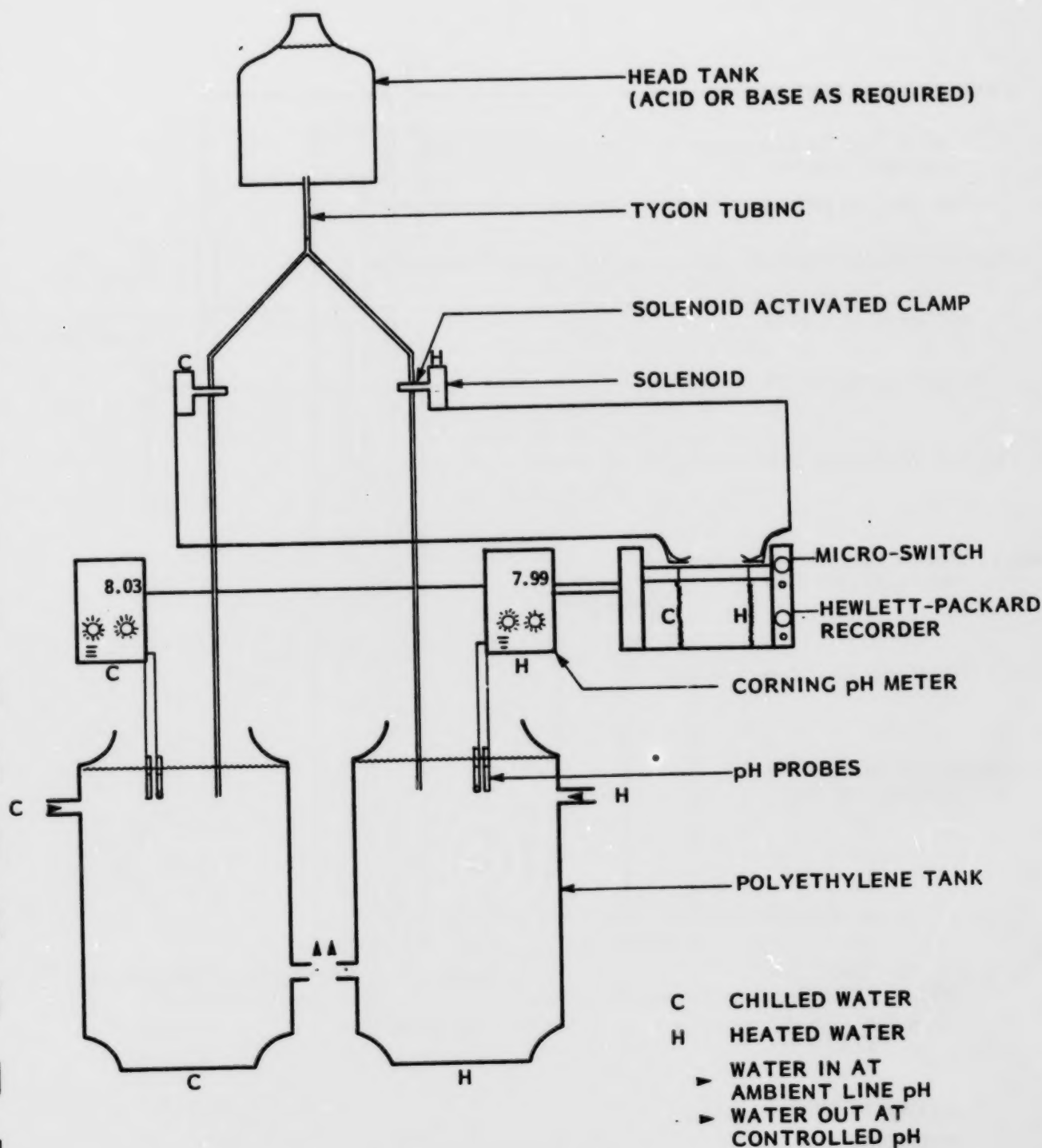


FIGURE 1
pH CONTROL SYSTEM

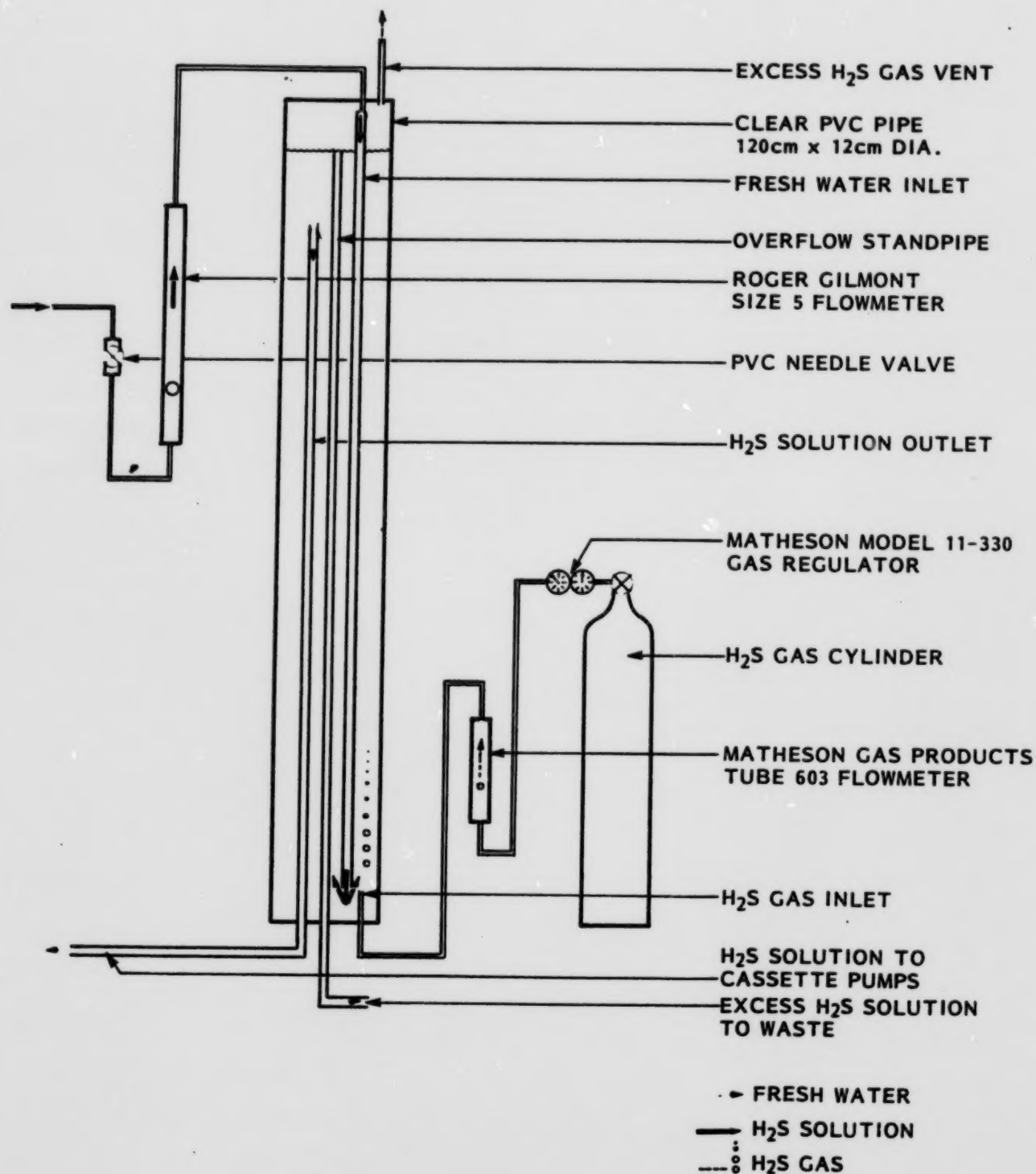


FIGURE 2
HYDROGEN SULPHIDE SOLUTION GENERATOR

bottom of the mixing cylinder. Hydrogen sulphide gas flow was controlled by a Matheson Model 11-330 regulator and a Matheson Tube Flowmeter (Model 603).

As the H_2S gas and inflow water rose in the tube, the gas was dissolved to form a concentrated solution. Gas flow and water flow rates were adjusted to provide the desired stock concentration. Excess H_2S solution was removed by a 115 cm standpipe overflow. Any excess H_2S gas was removed by a vent at the top of the mixing cylinder.

Hydrogen sulphide stock solution was removed from the mixing cylinder by a standpipe about 15 cm below the overflow. This standpipe led to a distribution manifold which supplied two multi-channel peristaltic pumps used to deliver stock solution to each bioassay tank.

D. H_2S Delivery System

A distribution system was constructed to deliver varying amounts of concentrated H_2S solution to each test tank (Figure 3). Two PVC manifolds were connected to the H_2S generator overflow. Manogon tubing attached to the manifolds passed through Manostat peristaltic cassette pumps which controlled flow rates to the test tanks. Two Manostat pumps were used. Each could simultaneously operate up to 10 cassettes. By varying tubing size, pump speed and cassette orientation (resulting in a doubling of pump speed) various delivery rates of H_2S stock solution could be obtained. Downstream of the cassette pumps, the H_2S stock solution was delivered to individual test tanks through 0.47 cm ID Tygon tubing. The H_2S stock solution was mixed with the metered dilution water flow prior to entry to the test tanks.

E. Test Tanks

Dilution water was delivered to the polyethylene test tanks by gravity flow through a PVC manifold and Tygon tubing from the constant-head tanks (Figure 4). Water level in the head tanks was regulated by float valves. Dilution flows to the test tanks were regulated with PVC needle valves and Roger Gilmont Size 5 flowmeters. Flow rate was averaged at 420 mL per minute. Two

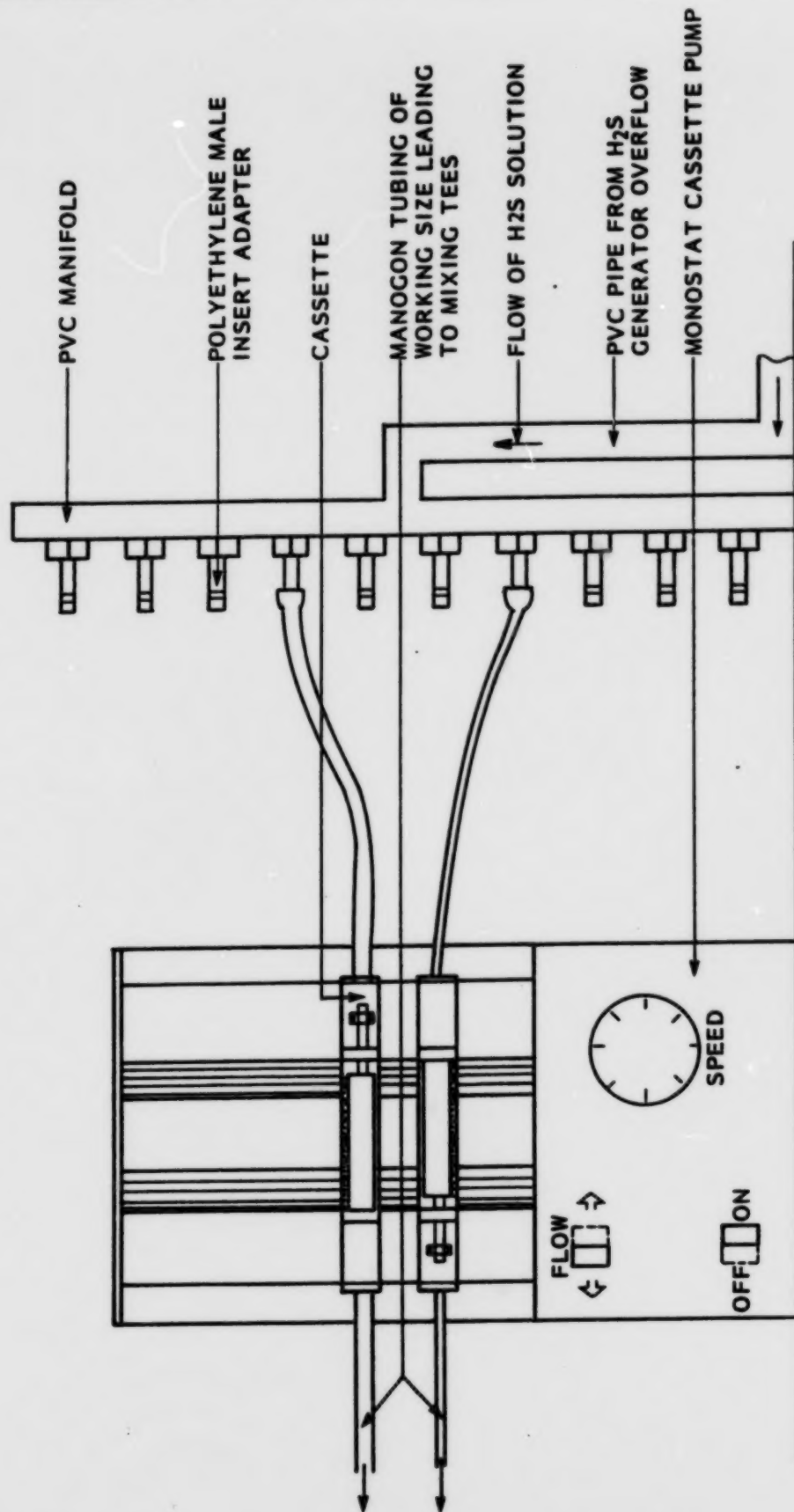


FIGURE 3
H₂S SOLUTION DELIVERY SYSTEM

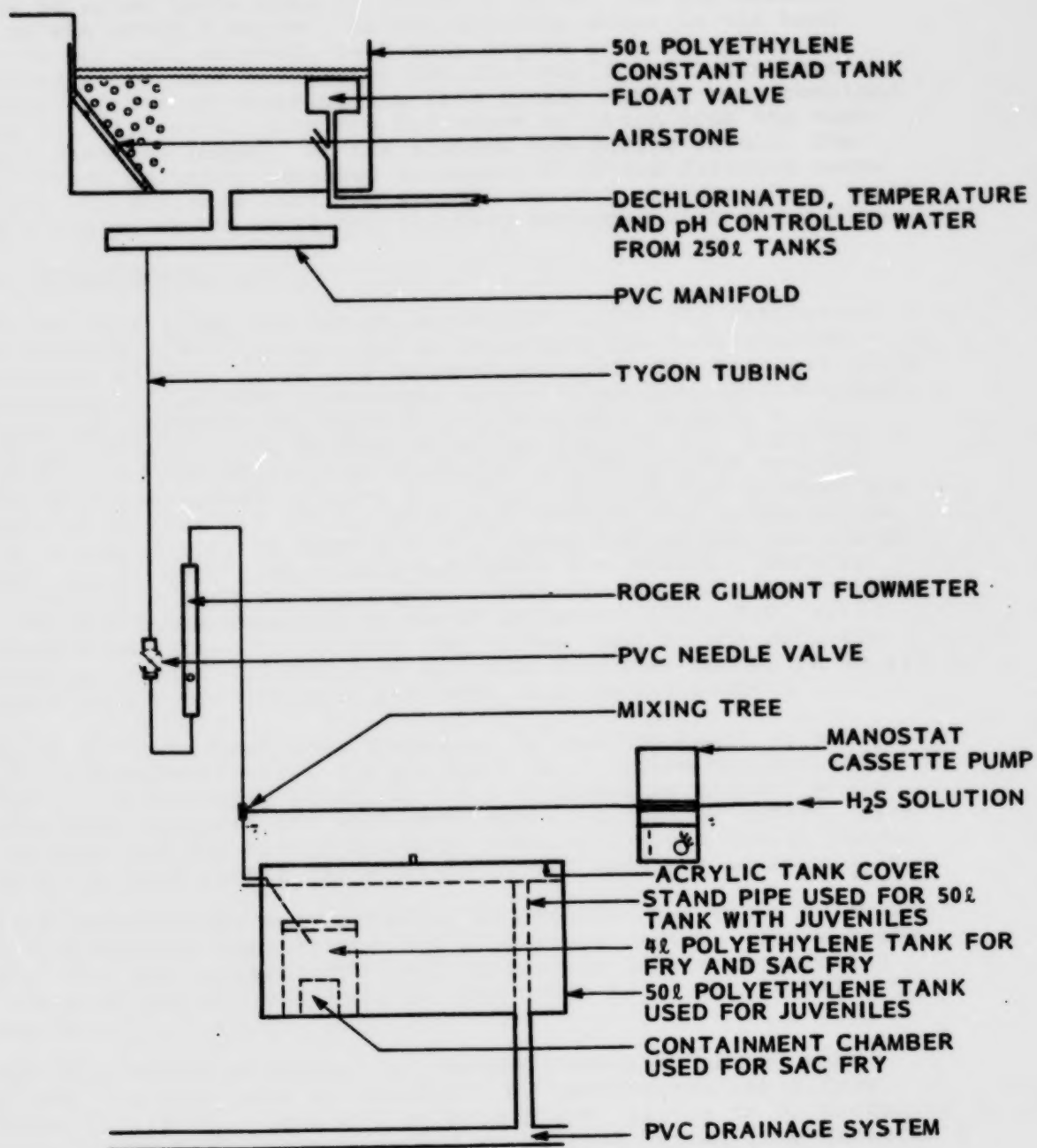


FIGURE 4
BIOASSAY APPARATUS

test tanks were used. For the 4 liter tanks used in sac-fry tests, the replacement time was about 10 minutes, while for the 50 liter tanks used in juvenile tests, the replacement time was about 2 hours. As the dilution water in the head tanks was well aerated, test tank oxygen concentrations remained at or near saturation for both small and large test tanks. Prior to entering the test tanks, mixing tees combined the dilution water with the H_2S stock solution from the Manostat cassette pumps. At the highest H_2S dosing levels, the H_2S stock solution amounted to about 1% of the dilution water flow. To minimize handling stress, sac-fry used in these tests were contained in small PVC chambers screened with 363 μ Nitex.

3. Experimental Design

The present study had two purposes: to verify the results of MacLaren's (1977) study, and to determine the separate and combined effects of pH and temperature (over the seasonal range expected at the BHWP discharge) on H_2S toxicity. A fractional factorial experimental design was employed. Basically, it consisted of a 3 x 3 factorial design rotated 45°, with the addition of two additional controls at extreme high and low temperatures tested (Figure 5). Thus, this design allowed the testing of four H_2S levels plus controls at five temperatures, but required only 11 test points. Three concurrent replicates were run at each temperature- H_2S condition tested. Usually 10 fish were used per replicate. The experimental design shown in Figure 5 was repeated at three pH levels (7.5, 8.0, 8.5). These were chosen to include the values used in the MacLaren experiments (pH 7.5 and 8.0) and also included higher pH levels occasionally occurring at the BHWP site during summer.

While it would have been desirable to conduct tests at all pH levels simultaneously, (or at least in a random manner) this was not technically possible, as individual pH control would have been required for each test tank. As the pH control system modified the entire bioassay flow to a given level, three separate experiments (at each pH level) were run sequentially.

Test temperatures were arranged to consider the known tolerance limits of each species and the life stage tested. The extreme high and low temperatures used approached (where technically feasible) but did not include, the upper and lower lethal limits.

Hydrogen sulphide levels (in terms of total sulphide) were chosen for each test on the basis of results available from MacLaren (1977). However, in some cases, little or no mortality occurred at H_2S levels expected to be 100% lethal from MacLaren's results. In these cases, tests were repeated at higher H_2S levels until substantial mortality occurred. Where possible, H_2S dose levels were arranged to result in nearly 100% mortality at the highest level and little or no mortality at the lowest H_2S level.

Both 1 hour and 96 hour bioassays were run. Temperature and pH levels for a given species remained constant for both bioassays,

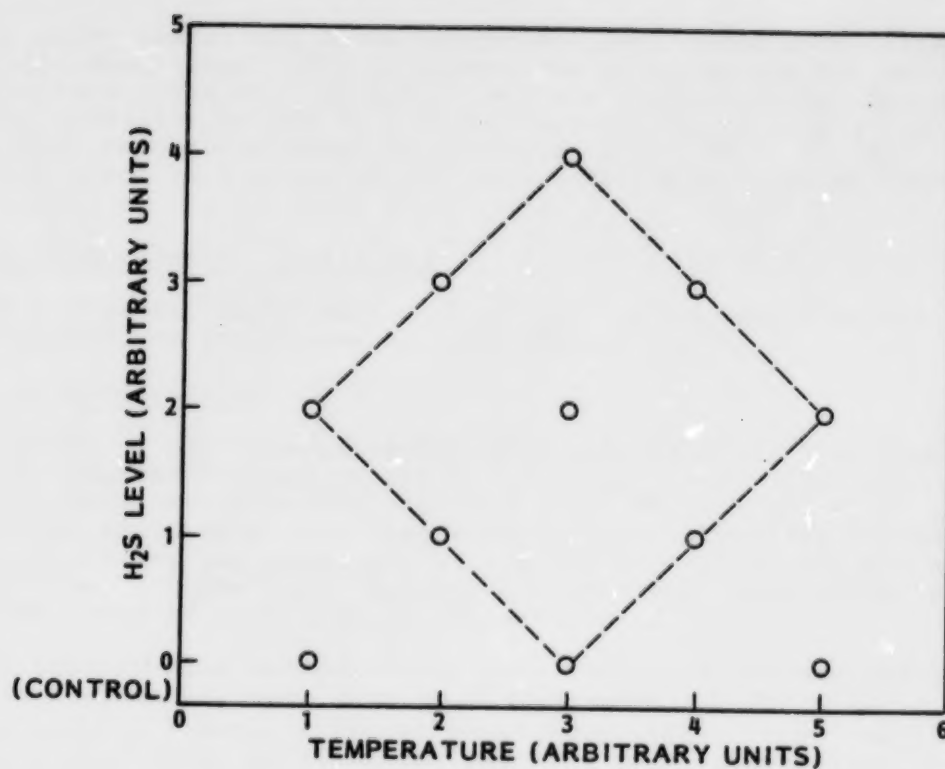


FIGURE 5
EXPERIMENTAL DESIGN FOR H₂S BIOASSAY STUDIES
- 3 REPLICATES RUN AT EACH H₂S/TEMPERATURE COMBINATION
- EACH EXPERIMENT REPEATED AT pH 7.5, 8.0, 8.5

but much higher H_2S levels were required to obtain mortality in the 1 hour bioassays.

In these tests, the three concurrent replicates were tested in individual tanks, each with separate dilution and H_2S metering controls. The only exception was the control tests, for which all replicate groups at a given temperature were held (in individual mesh containers) in a single tank. Thus, for an experiment at a given pH, 27 test tanks were operated simultaneously.

4. Experimental Procedures

Each bioassay experiment was carried out according to the standardized procedures outlined below.

A. Acclimation

Sac-fry of all species tested were acclimated to both experimental temperatures and pH prior to the bioassay. The length of this acclimation period was 72 hours. While a longer acclimation period would have been desirable to assure complete thermal acclimation, the shortness of the sac-fry period did not allow extended acclimation. Acclimation generally took place in the head tanks of the experimental apparatus.

As the juvenile rainbow trout were too large for acclimation in the head tanks, these fish were thermally acclimated in separate 1.2 meter diameter fiberglass tanks. The juveniles were acclimated at least two weeks at test temperatures prior to testing, at ambient line pH, which averaged pH 7.54 for the 1 hour bioassays and pH 7.49 for the 96 hour bioassays.

One group of rainbow trout fry (2 to 4 cm in length) used for preliminary 1 hour bioassay tests was not acclimated prior to testing. These fish were held at ambient line pH and a temperature approximating 12°C prior to testing. Results from this preliminary test series will also be included in this report.

B. One Hour Bioassays

The bioassay apparatus was operated at the required pH and test temperatures for at least 3 days prior to a test. During this time, the fish (sac-fry) were acclimated in the head tanks. On the day prior to the test, the H_2S delivery system was turned on and adjusted to provide the required hydrogen sulphide levels to the test tanks. The following morning, H_2S levels were measured and final adjustments made to assure the desired dosing levels. Generally, H_2S concentrations in the test tanks were "fine-tuned" by adjusting the dilution water flows, as the length of H_2S delivery tubing and slow pumping rates of the stock solution prevented rapid adjustment of H_2S delivery rates.

Acclimated sac-fry were removed from the head tanks and counted into containment chambers. As each replicate was placed into the test tanks, the time was noted. After 60 minutes the fish were removed. Fish mortality, temperature, pH and total H_2S concentration were determined for each test tank. The criterion for mortality of sac-fry was absence of heart beat (determined visually with an Olympus dissecting microscope). For fry and juveniles the heart was not visible. Hence, the criterion for mortality was the absence of reaction to stimulation by prodding and the lack of opercular movement.

Temperature was measured to the nearest one-tenth degree centigrade with a NBS guaranteed tolerance thermometer. A Corning Model 125 digital pH meter was used for individual test tank pH measurements. Three water samples were taken from each test tank for H_2S analysis.

C. Ninety-Six Hour Bioassays

Acclimation, apparatus start-up and test initiation procedures were similar to those used in 1 hour bioassays. In the 96 hour tests, mortality, temperature, pH and H_2S concentrations were determined twice daily (at 0800 hrs and at 1400 hrs) until the end of the experiment. Where necessary, adjustments were made in dilution water flow and/or H_2S delivery rates to maintain test tank concentrations at the desired levels.

5. H_2S Analysis

An automated continuous-flow colorimetric method of H_2S analysis was developed by Ontario Hydro's Analytic Services Section, using the Technicon Autoanalyzer II. The method was a modification of that outlined by the manufacturer (Technicon Publication No 112-81W) and allowed a minimum detection limit of 2 ppb total sulphide, with a reproducibility of between 0.2 and 1% over the 4 ppb to 100 ppb range (Ontario Hydro Research Division Report No 79-181-K). The method was an automated version of the colorimetric technique outlined in the Standard Methods (1975). Samples and standards were stabilized by the addition of cadmium to form insoluble cadmium sulphide which was suspended in solution with carboxy methyl cellulose. Samples were introduced to the analytical stream, where after acidification and reaction with p-aminodimethylaniline and ferric chloride, methylene blue was produced by the reducing action of H_2S . The methylene blue was measured colorimetrically at 660 nm. Outputs were recorded on a chart recorder calibrated twice daily with known standards. At each sampling period, three samples were analyzed from each tank. Each sample was sub-divided and three consecutive determinations were run (followed by a water blank). Results were averaged to determine the H_2S concentrations in each test tank at the time of sampling.

Hydrogen sulphide levels were measured as total H_2S (in ppb). These values were also converted to aqueous H_2S levels, using the formula of Clarke (1974), considering the temperature and pH values measured in each test tank at the time of sampling. For

these calculations, an ionic strength of 0.0033 was assumed to be typical for Great Lakes waters (Clarke, 1974).

6. Data Analysis

A. Time-Weighted Averages for 96 Hour Bioassays

Some fluctuations in temperature, pH and hydrogen sulphide concentrations occurred throughout the 96 hour experiments. As these parameters were measured twice per day (at 0800 hrs and at 1400 hrs) average values for each experiment were calculated on a time-weighted basis. For each day of the experiment, the period between 0800 and 1400 hrs was given a weighting of 0.25, and the period from 1400 hrs to 0800 hrs the following day was given a 0.75 weighting. These weighted average values were then used to compute mean temperature, pH and H₂S concentration for the entire experiment.

B. Response Surfaces

Percentage survival values obtained at each experimental pH were analyzed by response surface methods. In this analysis, first developed by Box (1956) and later modified to include parameter transformations by Lindsey et al (1970), experimental temperature and H₂S concentration were considered as two independent variables affecting survival of test fish. The computer program used for this analysis (described by Lindsey and Sandnes, MS 1972) was adapted for Ontario Hydro's UNIVAC computer (Ontario Hydro Research Division Report No 75-484-K). The program fits a second-order polynomial in transformed variables to the results:

$$y^{\delta} = b_0 x_0 + b_1 x_1^{\alpha_1} + b_2 x_2^{\alpha_2} + b_{11} x_1^{2\alpha_1} + b_{22} x_2^{2\alpha_2} + b_{12} x_1^{\alpha_1} x_2^{\alpha_2}$$

where:

y = calculated response (percentage survival)

$b_0 x_0$ = intercept value (where $x_1 = x_2 = 0$)

x_1 = test temperature

x_2 = H₂S concentration

$\delta, \alpha_1, \alpha_2$ = power transformations of the dependent and independent variable selected by an iterative procedure converging on those transformations giving a minimum residual variance.

The computer program fits both transformed and untransformed (where $\delta = \alpha_1 = \alpha_2 = 1$) models. The adequacy of these models is then assessed through Analysis of Variance and examination of residuals (deviations between observed and predicted values). Contour plots are also produced describing the effects of temperature and H₂S concentrations on survival. Separate contour plots were produced for each of the three experimental pH levels tested. Each data set was analyzed in terms of both total H₂S concentrations and aqueous (undissociated) H₂S levels.

Although three concurrent replicates were run for each test condition, separate H_2S delivery systems were used for each replicate tank. This resulted in some variation (occasionally quite substantial) in H_2S concentrations among replicates. Thus, it was decided to input results from each test tank separately in the response surface analysis. This resulted in the loss of the "lack of fit" term in the Analysis of Variance, but generally provided a response surface model which better described the observed data, as the "replicates" at higher H_2S concentrations often spanned a considerable range of mortality levels. Thus, most response surface models were based on 33 separate data points. Some tests were partially or completely repeated where highest H_2S levels did not cause substantial mortality in the first test, and all available data were combined to produce the response surface model. The greatest number of data points used in an individual model was 99 (where a test was repeated three times).

RESULTS

1. One Hour Bioassays

A. Rainbow Trout Sac-Fry (Group 1)

The first group of rainbow trout sac-fry was tested in the spring of 1979. Tests were conducted at pH 7.5, 8.0 and 8.5. Test temperatures ranged from 4°C to 22°C (except one test at pH 7.5 which used test temperatures as high as 24°C). Experimental conditions and survival data for each test are listed in Appendix 2A.

The first rainbow trout sac-fry test, conducted at pH 7.5, did not result in substantial mortality at the highest H_2S levels tested. Consequently, this test was repeated, using higher dosing levels. Results for both tests were combined (72 data points) to fit the response surface model shown in Figure 6A. In this figure, response isopleths are shown for mortality levels of 10%, 50% and 90% mortality, designated by LC (lethal concentration) 10, 50 and 90. Contours are shown as solid lines within the experimental design. Dashed lines represent extrapolation of the regression model, shown here to display the general form of the model.

Analysis of Variance (Appendix 2B) indicates that both temperature (x_1) and H_2S concentration (x_2) significantly affected rainbow trout sac-fry survival. Examination of residuals (difference between observed data and values predicted by the model) provides an indication of model error. For the 72 test points used to fit the model in Figure 6A, the mean residual amounted to 6.86%.

In Figure 7, a transect of the regression model is compared with observed data at 14°C, the centre of the experimental design (where the greatest number of data points were concentrated). The model agrees quite well with the observed data, but is not capable of following the abrupt transition from complete survival

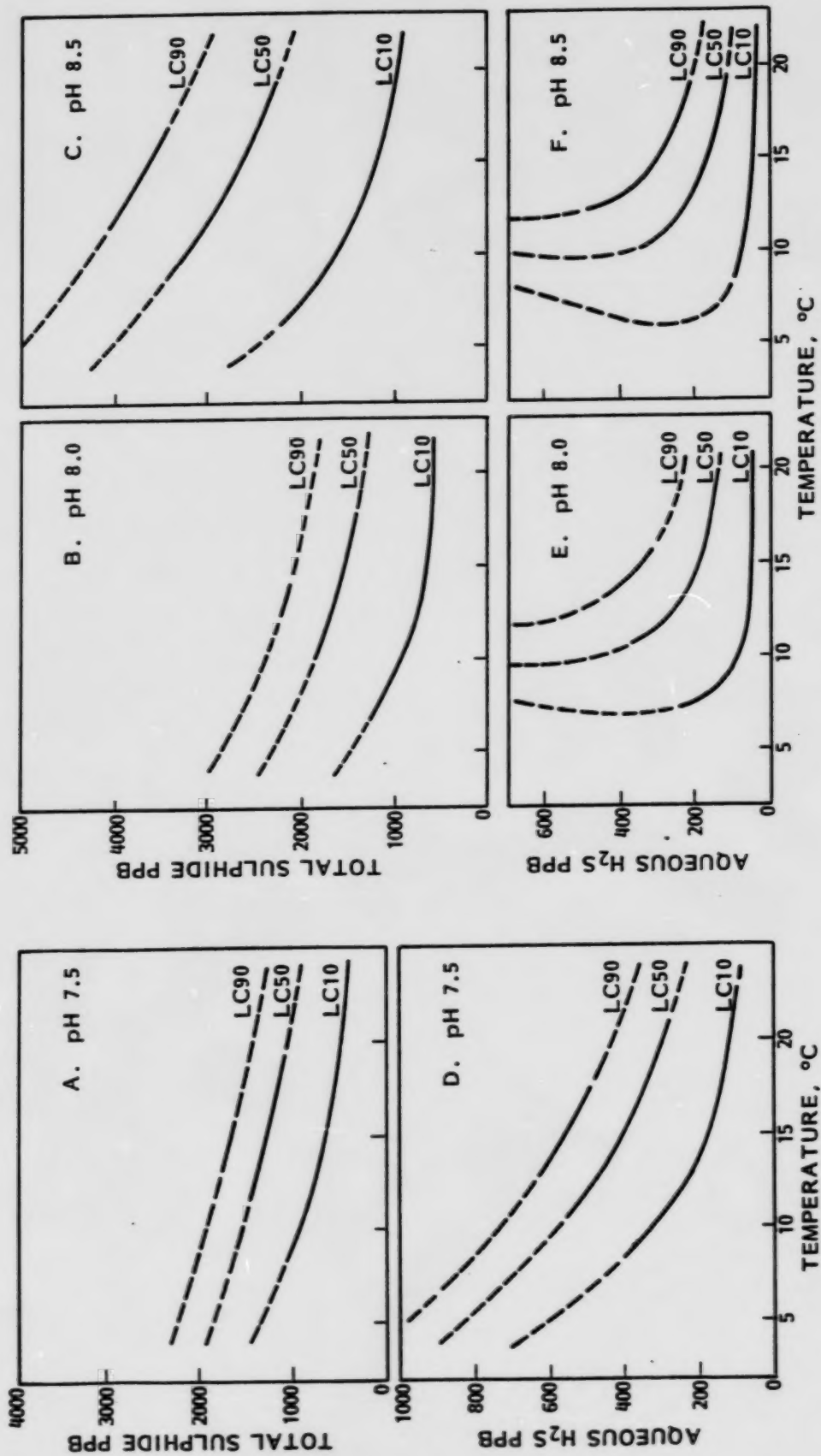


FIGURE 6
RAINBOW TROUT SAC-FRY (GROUP 1)
 H_2S BIOASSAY - 1 HOUR

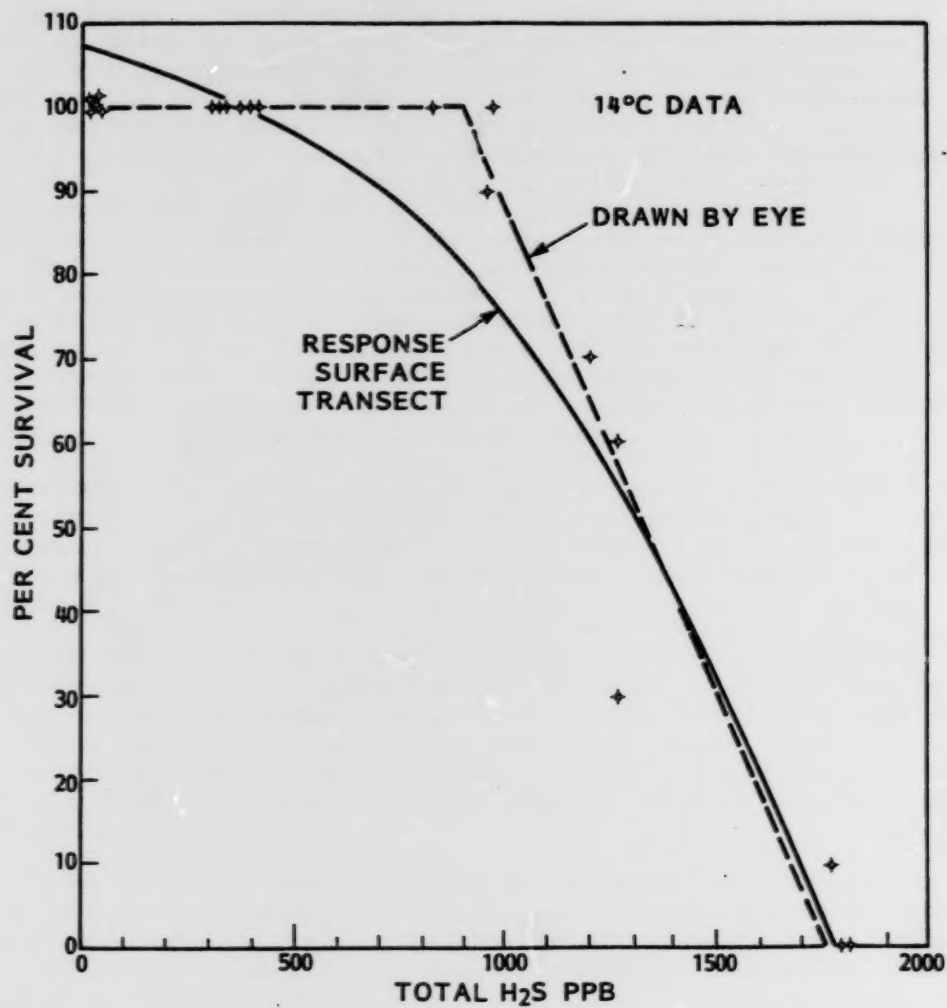


FIGURE 7
COMPARISON OF OBSERVED SURVIVAL DATA
WITH A TRANSECT FROM RESPONSE SURFACE MODEL.
DATA FOR RAINBOW TROUT SAC-FRY (GROUP 1)
IN 1 HOUR BIOASSAY

to increasing mortality which occurred over the 800 ppb to 1000 ppb range. Examination of regression models fitted to the other tests in this study suggests that the comparison in Figure 7 is typical for most tests. Hence, reliance should not be placed on the 100% survival contour estimated by the regression models. The 90% survival contour (LC10) probably provides a reasonable indication of test conditions which will first begin to cause some mortality. The LC50 predicted by the model is in good agreement with the observed data, as is generally the case with most tests conducted. While predicted H_2S levels yielding total mortality agree closely with observed values in this example, it is felt that in most cases, the predicted LC10 to LC90 range is a good estimator of the range of H_2S values over which mortality occurs. Thus, LC10, LC50 and LC90 values will be shown for each test in the presentation of results, with the greatest reliance being placed upon the LC50 value.

Results for experiments conducted at pH levels of 8.0 and 8.5 are shown in Figures 6B and 6C. Analysis of Variance for these regression models (Appendices 2C, 2D) again indicates that both total H_2S concentration and temperature significantly affected survival.

At all pH levels, rainbow trout sac-fry were more sensitive to given levels of H_2S as temperature increased, despite the fact that at the higher temperatures less of the total sulphide concentration was in the most toxic (aqueous) form. Presumably, the increased toxicity with temperature is a function of more rapid H_2S uptake resulting from higher metabolic rate at warm temperatures.

Figures 6A to 6C demonstrate that with increasing pH over the 7.5 to 8.5 range, progressively higher concentrations of total H_2S are required to produce a given level of mortality. Primarily this is a function of the dissociation characteristics of H_2S over this pH range.

In order to determine whether the change in toxicity to H_2S with pH was entirely due to the degree of dissociation (ie, whether aqueous H_2S was the only toxic form), results of the three bioassay tests were re-analyzed in terms of aqueous H_2S levels. Contour plots of the fitted models are shown in Figures 6D to 6F. The regression models, Analysis of Variance and mean residuals are listed in Appendices 2E to 2G. As two of the models (Figures 6E and 6F) show abrupt upward curvature of survival contours at the low temperature boundaries of the experimental design, extrapolation of these models should be avoided. When results are compared within the experimental design (solid contours) it is evident that the fish are somewhat more sensitive to a given level of aqueous H_2S as pH increases. In this example, the greatest difference occurs over the pH 7.5 to pH 8.0 range. Also, the increased sensitivity with increased temperature that was noted in the total H_2S analysis is strongly evident for the H_2S aqueous models.

B. Rainbow Trout Sac-Fry (Group 2)

The second group of rainbow trout sac-fry was tested early in 1980. These tests were conducted in the same general time period as the 1 hour rainbow juvenile tests and the 96 hour sac-fry and juvenile tests (to be reported later in this section). Test temperatures for the Group 2 rainbow sac-fry experiments ranged from 4°C to 20°C. Experimental conditions and survival data for each test are listed in Appendix 3A. Response surface models fitted to these results and associated Analyses of Variance are listed in Appendices 3B to 3G.

Contour plots displaying the LC10 to LC90 values are shown in Figure 8. Results are generally comparable to those obtained for the Group 1 rainbow trout tests, although the Group 2 fish displayed somewhat greater resistance at all pH levels (compare LC50's for total H_2S in Figures 6 and 8). Again, progressively greater amounts of H_2S were required to produce mortality over the pH 7.5 to pH 8.5 range (Figures 8A to 8C). The analysis for aqueous H_2S (Figures 8D to 8F) suggests an increased sensitivity with increasing pH, but this is less marked than in the previous (Group 1) tests. Between pH 8.0 and pH 8.5, the Group 2 fish were more sensitive to given aqueous H_2S levels only at warm temperatures (>15°C). However, it should be noted that due to asymmetry in the experimental design (the design was symmetrical with respect to total H_2S levels) little data is available for low temperature/high H_2S levels when the aqueous form is considered.

C. Rainbow Trout Fry (Unacclimated)

Results for rainbow trout fry tested for 1 hour periods in the spring of 1979 are listed in Appendix 4A. As previously indicated, these tests were done as a preliminary series to establish procedures. Fry were tested over the 5°C to 24°C range (lower temperatures were used for other rainbow tests). The fish were not acclimated to experimental pH or temperature prior to testing. They had been held at a pH near 7.5 and a temperature near 12°C prior to these tests.

Response surface models (and Analyses of Variance) fitted to the rainbow fry data are listed in Appendices 4B to 4G. The contour plots (Figures 9A to 9C) again indicate that progressively greater total H_2S levels were required to produce a given mortality level as pH increased. While tests at pH 7.5 and pH 8.0 indicate significant mortality increases with increased temperature, the model in Figure 9C suggests a slight decline in mortality at warm temperatures at the highest (8.5) pH level. However, thermal effects were not significant for the pH 8.5 mortality model (Appendix 4D).

Analysis of aqueous H_2S data (Figures 9D to 9F) again indicated a trend towards greater toxicity of a given aqueous H_2S level as pH increased, although the trend was not consistent, ie, there was no apparent change between results for pH 7.5 and pH 8.0.

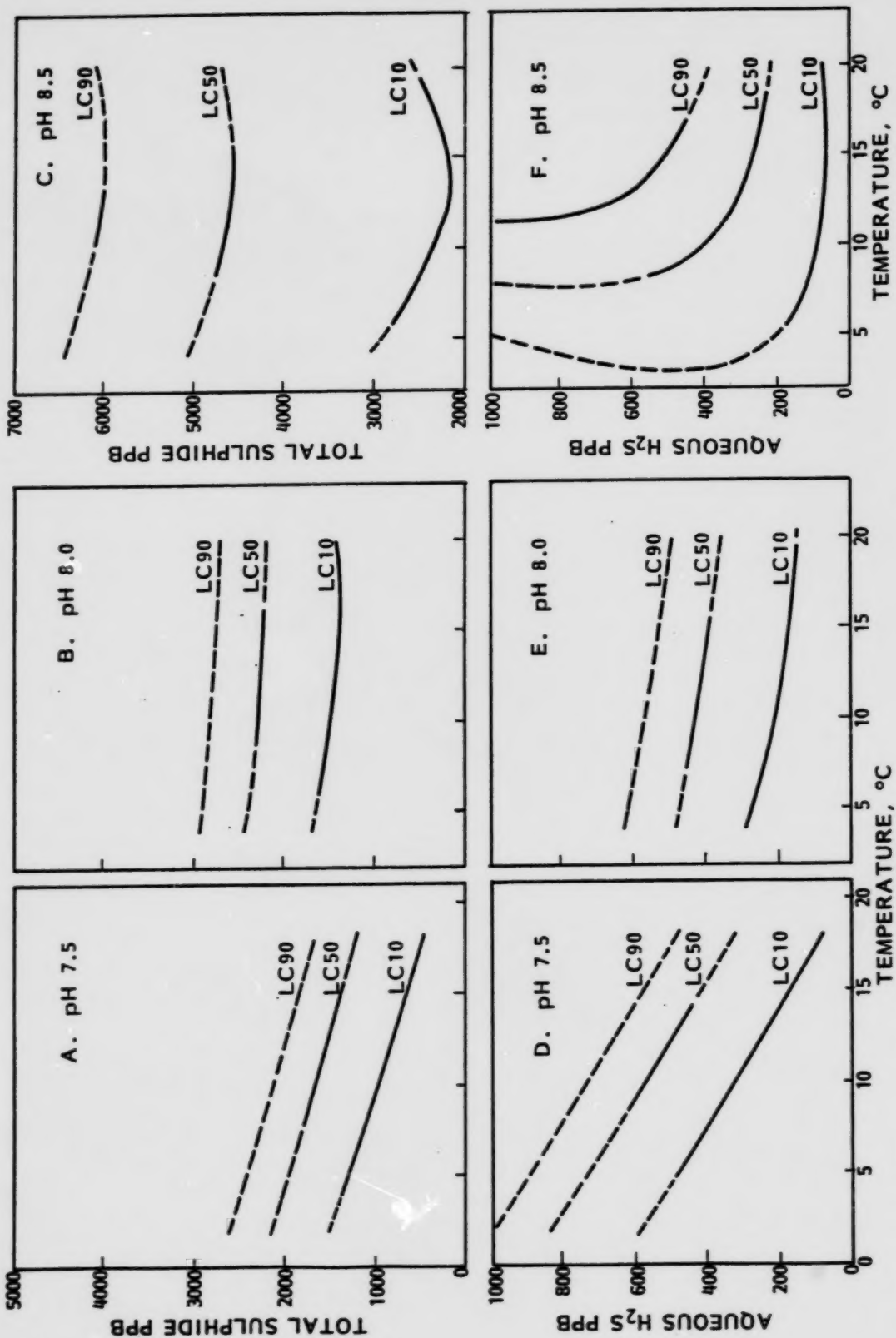


FIGURE 8
RAINBOW TROUT SAC-FRY (GROUP 2)
H₂S BIOASSAY - 1 HOUR

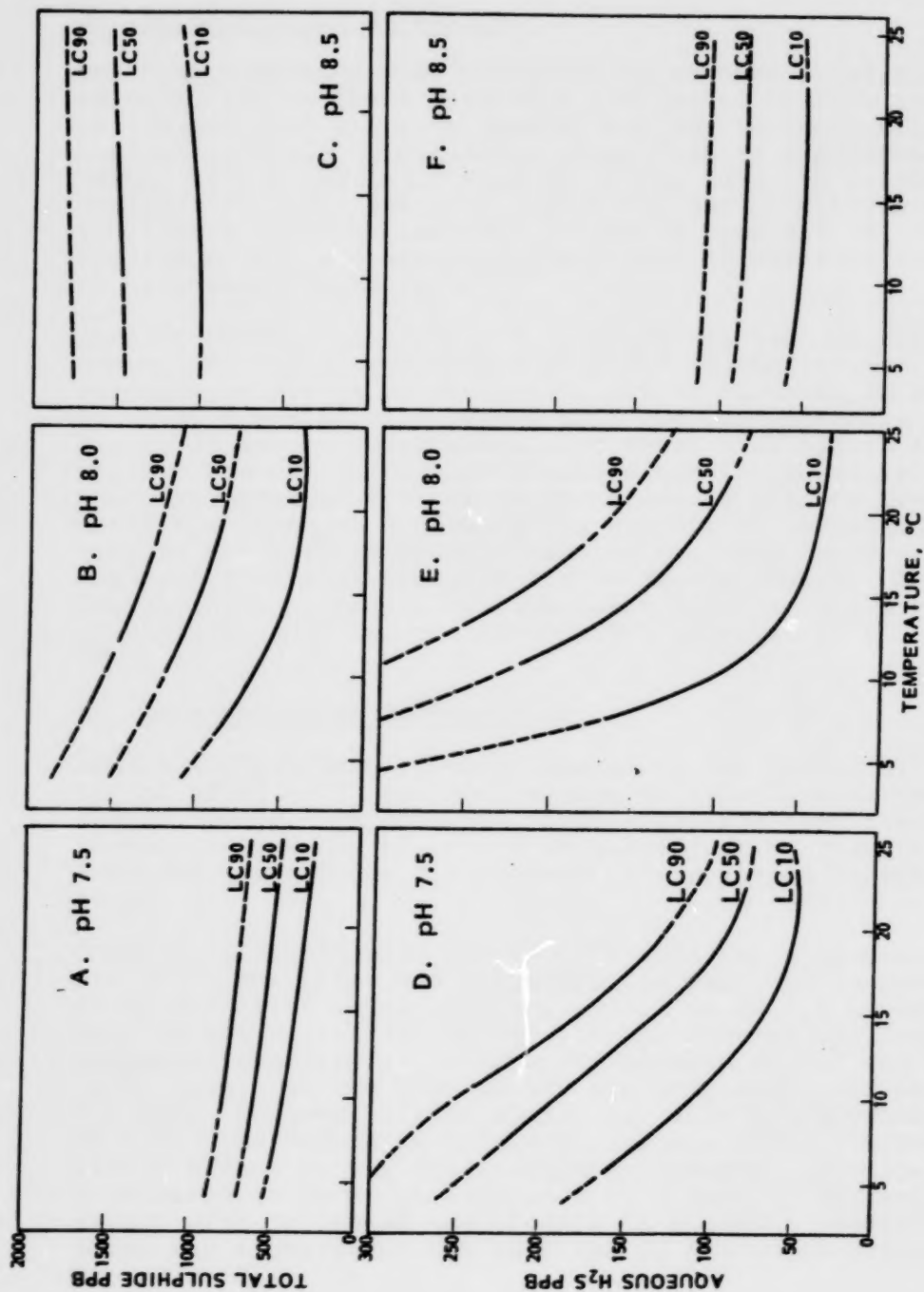


FIGURE 9
RAINBOW TROUT FRY (UNACCLIMATED)
H₂S BIOASSAY - 1 HOUR

Again, thermal effects were significant for the pH 7.5 and pH 8.0 tests, but were not significant for the pH 8.5 test (Appendices 4E to 4G).

D. Rainbow Trout Juveniles

One hour bioassays were conducted on rainbow trout juveniles in early 1980. These fish were acclimated to each experimental temperature prior to testing but due to their size, it was not possible to acclimate these fish to experimental pH levels before testing. Mean pH in the juvenile rainbow trout acclimation tanks was 7.54. Experimental conditions and survival data for each test are listed in Appendix 5A. Response surface models and associated Analyses of Variance are listed in Appendices 5B to 5G.

Contour plots of the rainbow juvenile results (Figure 10) again indicate a progressive decrease in sensitivity to total sulphide levels as pH increased, but an increase in sensitivity to aqueous H_2S with increased pH. The LC50 contours in Figure 10 are quite comparable to those obtained in the Group 1 rainbow sac-fry tests, but indicate greater sensitivity to H_2S than was observed for the second group of rainbow trout sac-fry. While the juvenile contour plots suggest an increased sensitivity to H_2S with increased temperature, this trend was less apparent than that observed in the sac-fry tests. For the models shown in Figure 10, thermal effects on survival were significant only for the aqueous H_2S tests at pH 8.0 (Appendix 5F).

E. Lake Whitefish Sac-Fry

Lake whitefish sac-fry were hatched in the spring of 1979 and tested after a 72 hour acclimation to experimental temperatures and pH levels. Experimental conditions and test results are listed in Appendix 6A, while models fitted to results and Analyses of Variance for the models are listed in Appendices 6B to 6G.

Contour plots (Figures 11A to 11C) indicate a progressive increase in the total H_2S concentration required to produce a given mortality level over the pH 7.5 to pH 8.5 range. When results are considered in relation to aqueous H_2S , substantially increased sensitivity is apparent between pH 7.5 and pH 8.5 (LC50 levels at the high pH are approximately half those at the low pH). However, little change was apparent between pH 7.5 and pH 8.0. The models in Figure 11 suggest that temperature had little effect on the total sulphide results. When data are considered in terms of aqueous H_2S , a general increase in sensitivity with increased temperature is evident. However, this trend was significant for only the pH 8.5 model (Figure 11F, Appendix 6G).

In general, lake whitefish sac-fry appear substantially more sensitive to H_2S than either rainbow trout fry or juveniles. The lake whitefish LC50 values are approximately 50% of those observed for the rainbow trout over most experimental conditions.

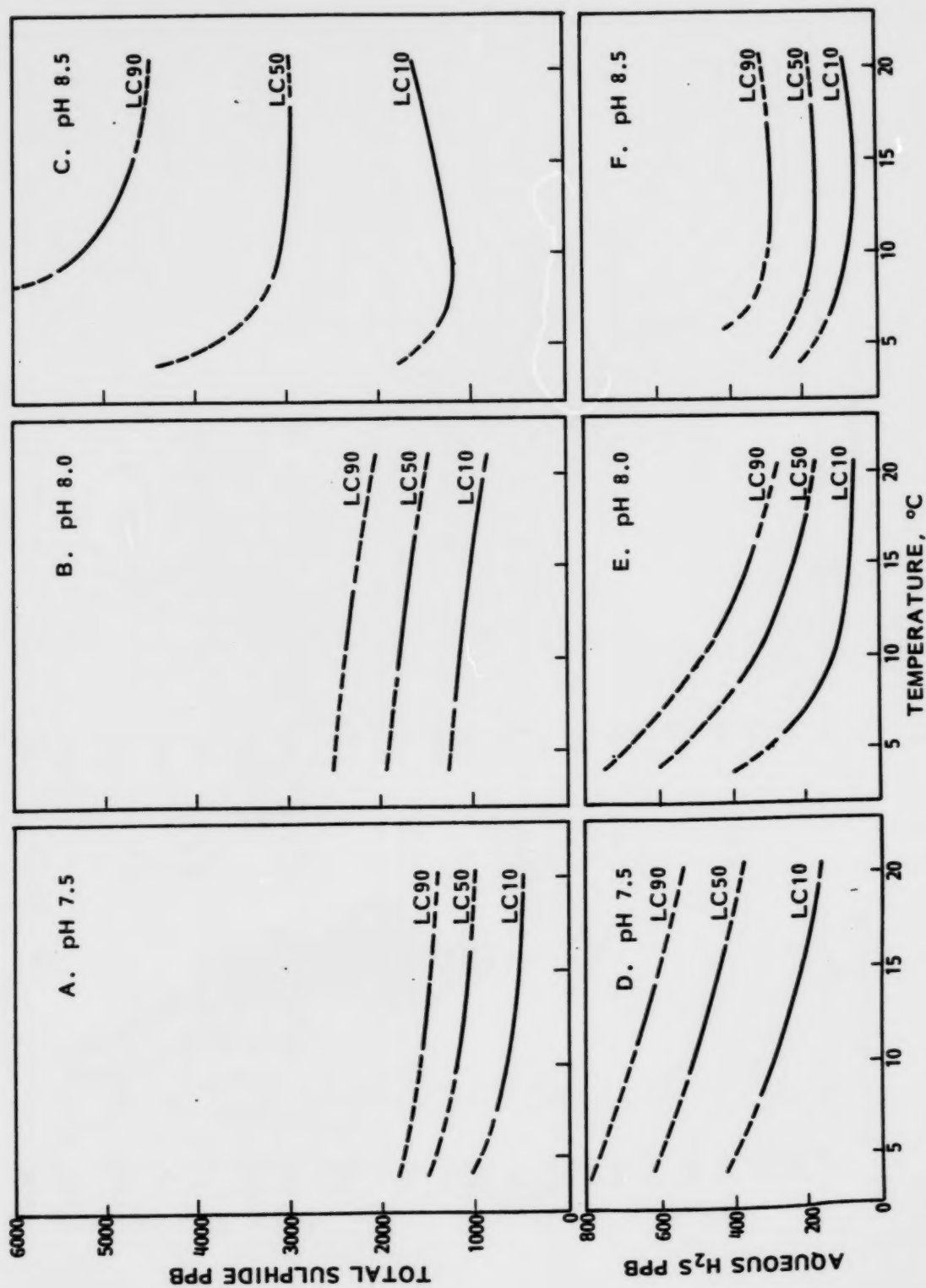


FIGURE 10
RAINBOW TROUT JUVENILES
H₂S BIOASSAY - 1 HOUR

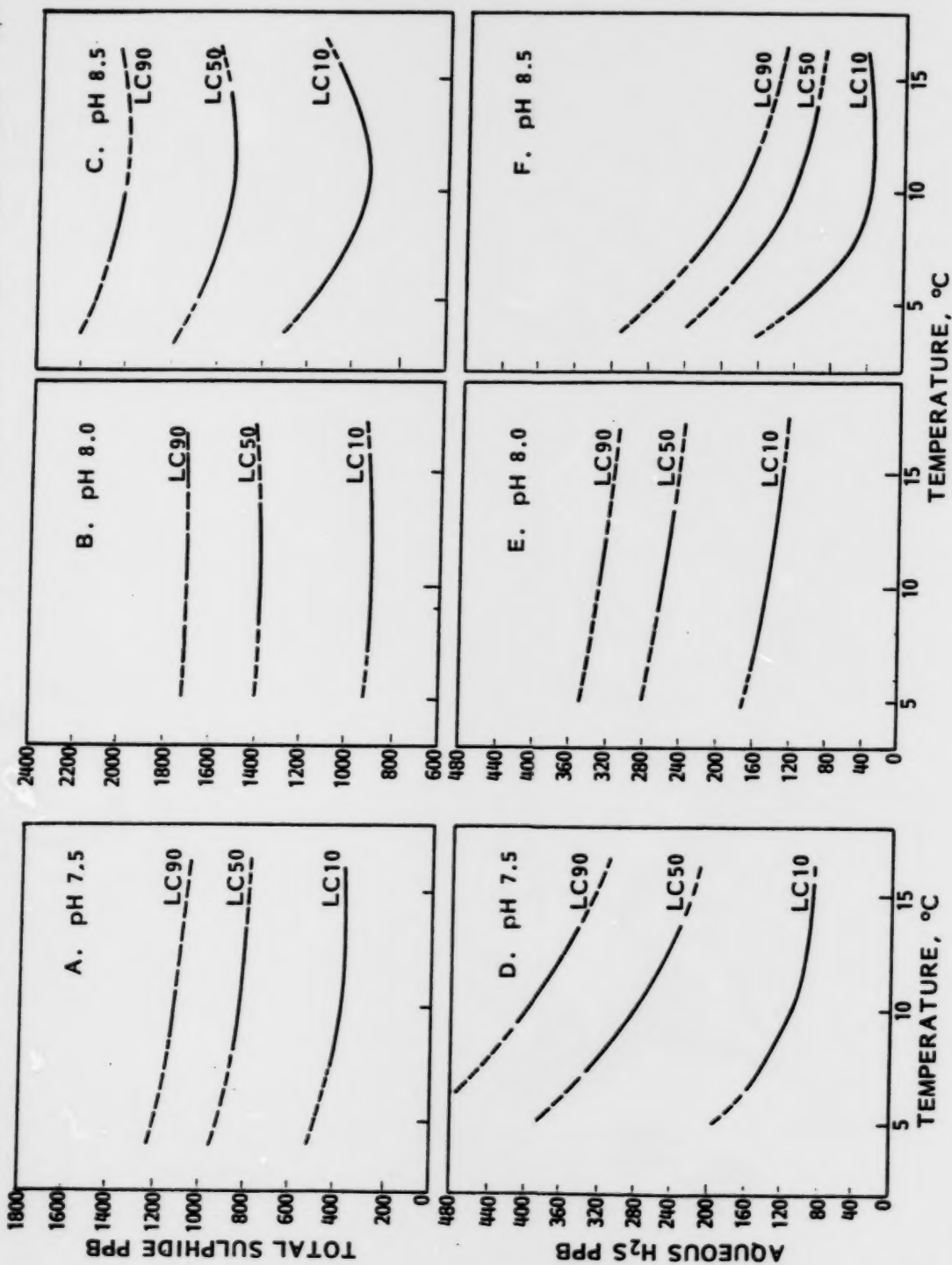


FIGURE 11
LAKE WHITEFISH SAC-FRY
 H_2S BIOASSAY - 1 HOUR

F. Round Whitefish Sac-Fry

Round whitefish sac-fry were tested concurrently with the lake whitefish in the spring of 1979. Fry were acclimated to experimental pH and temperatures for 72 hours (in head tanks) prior to testing. Test results are listed in Appendix 7A. Models fitted to the results and Analyses of Variance are listed in Appendices 7B to 7G. The contour plots of the round whitefish models (Figure 12) indicate that substantially greater total H_2S levels are required to produce a given mortality over the pH 7.5 to pH 8.5 range, while lower aqueous levels are required over this range. However, the results in Figure 12 do not indicate a linear progression with respect to pH, as results of the total sulphide analysis at pH 7.5 and pH 8.0 are little different. When the aqueous H_2S models are considered, little change in sensitivity is apparent between pH 8.0 and pH 8.5. All models in Figure 12 indicate an increased sensitivity to H_2S with increased temperature. This effect is most evident for the aqueous H_2S models (Figures 12D to 12F) where a significant thermal component was observed for each model (Appendices 7E to 7G).

G. Rock Bass Sac-Fry

Rock bass sac-fry were tested in the early summer of 1979. These fish were produced from newly fertilized eggs collected in Baie du Dore, Lake Huron and incubated in the laboratory. Acclimation to experimental pH and temperatures was done in the head tanks for 72 hours prior to experimentation. Test results are listed in Appendix 8A. Models fitted to the results and Analyses of Variance for the models are listed in Appendices 8B to 8G.

Contour plots of the rock bass survival models (Figures 13A to 13C) indicate a progressive increase in the level of total H_2S required to produce a given mortality as pH increased from 7.5 to 8.5. Also, as pH increased, sensitivity to given aqueous H_2S levels also increased. Results for the tests at pH 7.5 and pH 8.0 indicate an increased sensitivity to H_2S with increasing temperatures. This was most apparent for the aqueous H_2S models (Figures 13D and 13E, Appendices 8E and 8F) where a significant thermal effect occurred.

However, rather unusual results were obtained in the pH 8.5 test, as survival was lowest at cold temperatures. As these results differ from all other tests conducted in this study, it is suspected that these data are in error. The low survival of pH 8.5 rock bass at cold temperatures is likely the result of prolonged exposure of these fish to adverse (cold) conditions. This group of fry was incubated at rather low temperatures (mean $10.6^{\circ}C$) to retard development in order that newly hatched fry would be available for the entire test series. In contrast, fry for the pH 7.5 and pH 8.0 tests were incubated at much warmer temperatures ($15.6^{\circ}C$ to $27.2^{\circ}C$) more representative of those normally experienced by rock bass eggs. It is suspected that continued acclimation of the pH 8.5 rock bass at cold temperatures was the cause of low survival in the bioassay tests. In contrast,

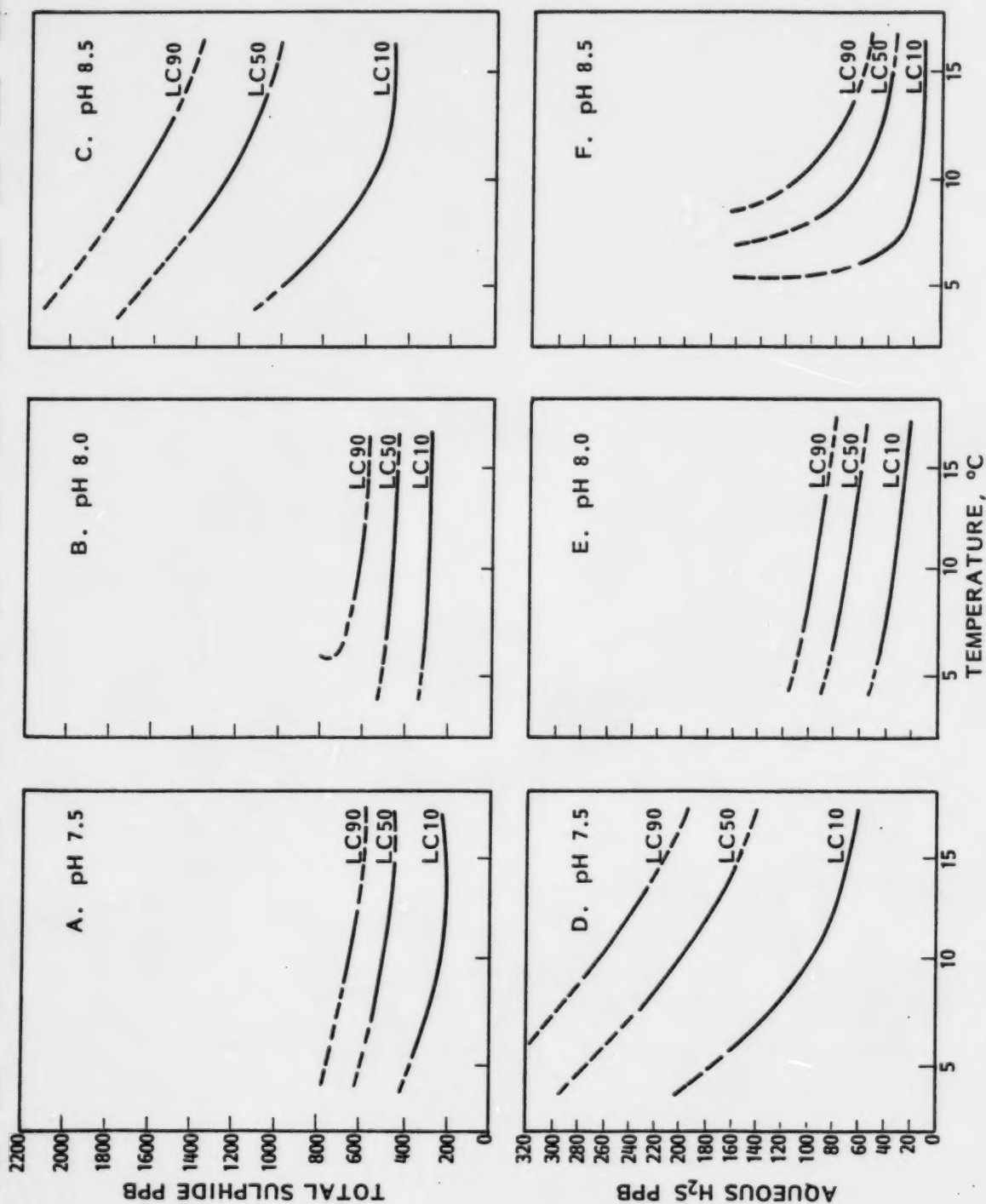


FIGURE 12
ROUND WHITEFISH SAC-FRY
H₂S BIOASSAY - 1 HOUR

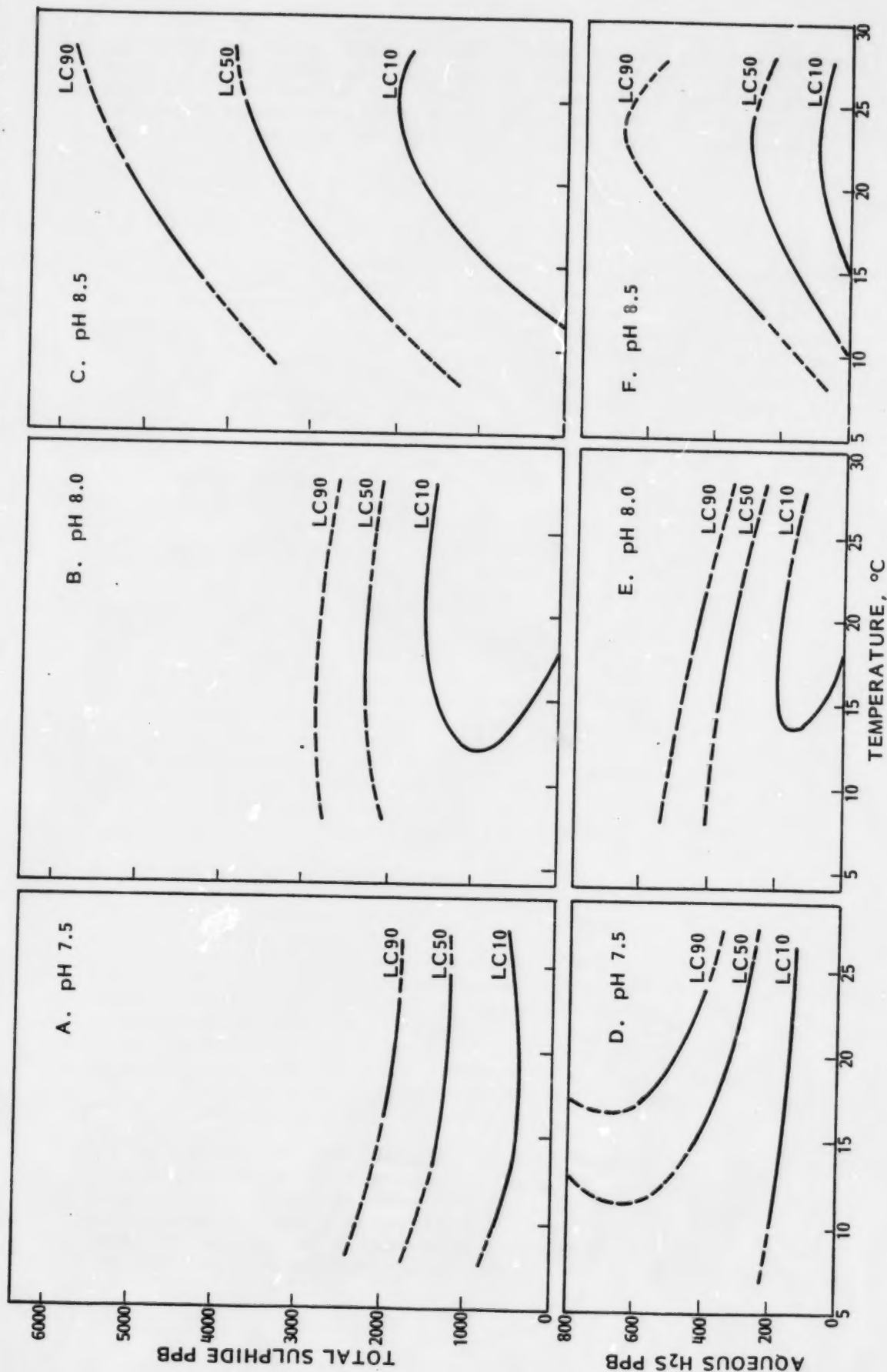


FIGURE 13
ROCK BASS SAC-FRY
H₂S BIOASSAY - 1 HOUR

survival of these fish at warm temperatures (following pre-test warm acclimation) was quite high. Thus, survival contours for the pH 8.5 rock bass tested at temperatures below approximately 20°C are likely erroneous, while results obtained at warmer temperatures appear more reasonable.

2. Ninety-Six Hour Bioassays

A. Rainbow Trout Sac-Fry

Rainbow trout sac-fry were tested in 96 hour bioassays during the early spring of 1980. These fish were from the same stock as those used for the "Group 2" one hour tests. These sac-fry were acclimated to experimental pH and temperatures for 72 hours prior to testing. Experimental conditions and test results are shown in Appendix 9A, while response surface models fitted to results and associated Analyses of Variance are listed in Appendices 9B to 9G.

As with the one hour bioassays, LC10, LC50 and LC90 contours have been plotted for the models obtained at each test pH, with separate models being calculated for total sulphide and aqueous H₂S (Figure 14). Over the range of pH 7.5 to pH 8.5, progressively higher levels of total sulphide were required to produce a given level of mortality (Figures 14A to 14C). Rainbow sac-fry tested at pH 8.5 were considerably more sensitive to given levels of aqueous H₂S at pH 8.5 than at lower pH levels, but little difference was apparent between the pH 7.5 and pH 8.0 aqueous H₂S models (Figures 14D to 14E). While there was a general trend toward greater sensitivity to H₂S at higher temperatures, this was most clearly defined in the aqueous H₂S models, where significant thermal effects were evident at all pH levels (Appendices 9E to 9G). While the general pattern of results obtained in the 96 hour bioassays was similar to that observed in the 1 hour tests, lethal levels of H₂S in the 96 hour bioassays were more than an order of magnitude (approximately 20 times) lower than those observed in 1 hour tests.

B. Rainbow Trout Juveniles

The 96 hour rainbow trout juvenile bioassays were conducted early in 1980, using fish from the same stock used in the 1 hour bioassays. As previously indicated, the size of these fish precluded pre-test acclimation in the bioassay apparatus head tanks. Thus, although the rainbow juveniles were thermally acclimated for periods from four to seven weeks in large tanks prior to testing, they were not exposed to controlled pH levels. Experimental conditions and tests results are shown in Appendix 10A, while response surface models and Analyses of Variance are shown in Appendices 10B to 10G.

Contour plots of the response surface models indicate a progressive decrease in sensitivity to total sulphide concentration with increased pH (Figures 15A to 15C). When results are considered in terms of aqueous H₂S levels, sensitivity to a given level of aqueous H₂S increased substantially with increased pH. The LC50 levels of H₂S_(aq) at pH 7.5 were approximately double

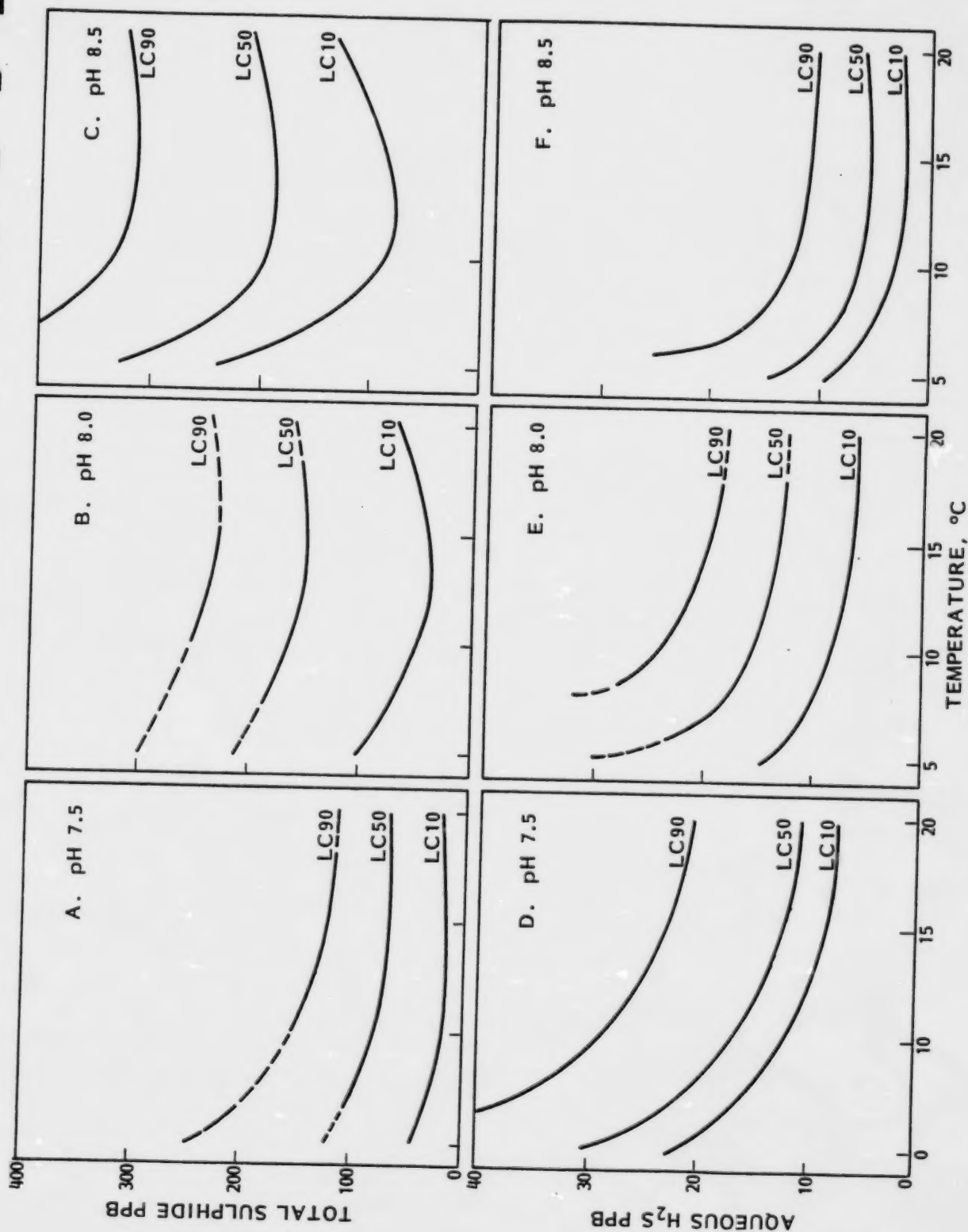


FIGURE 14
RAINBOW TROUT SAC-FRY H₂S BIOASSAY - 96 HOUR

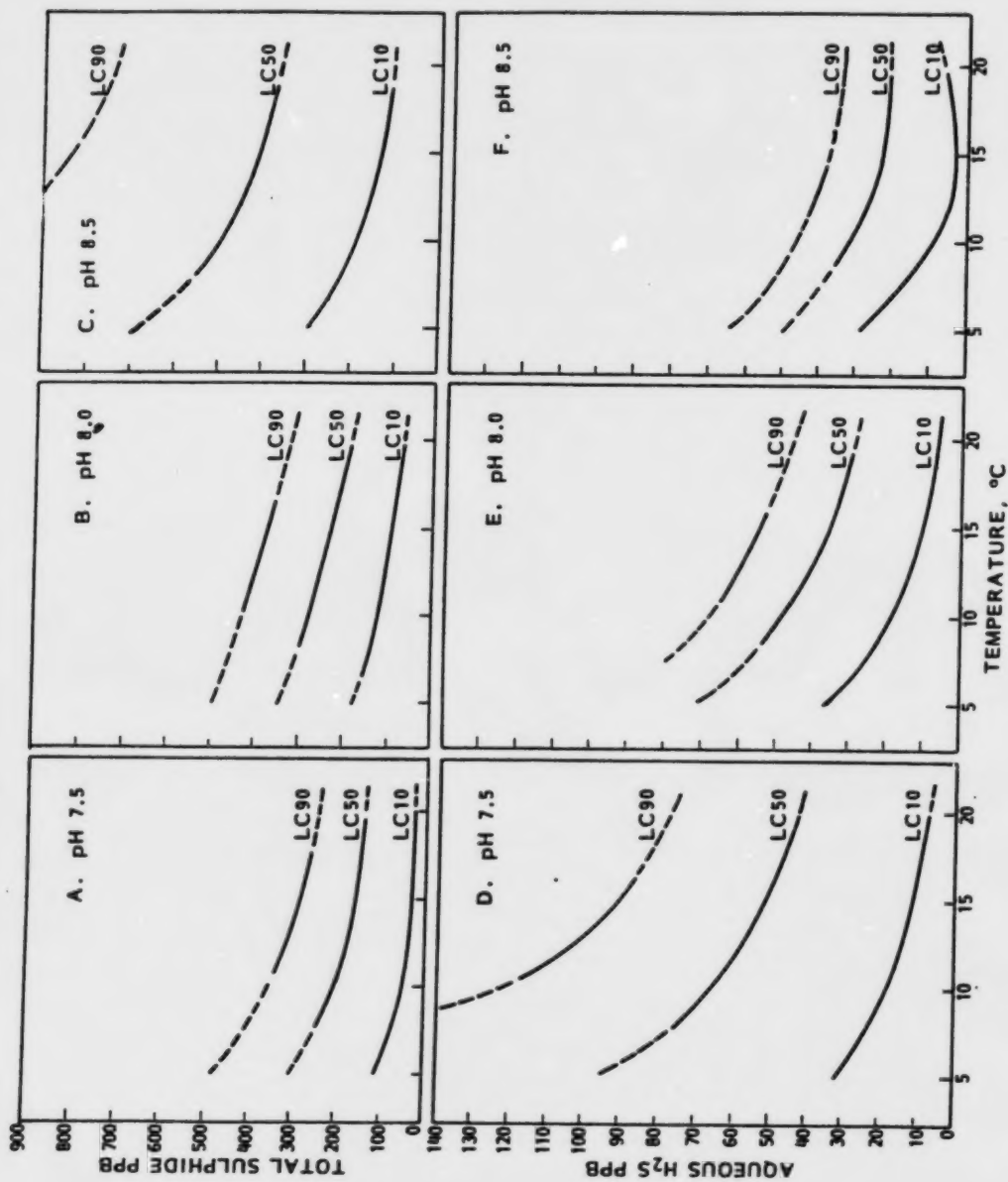


FIGURE 15
RAINBOW TROUT JUVENILES
 H_2S BIOASSAY - 96 HOUR

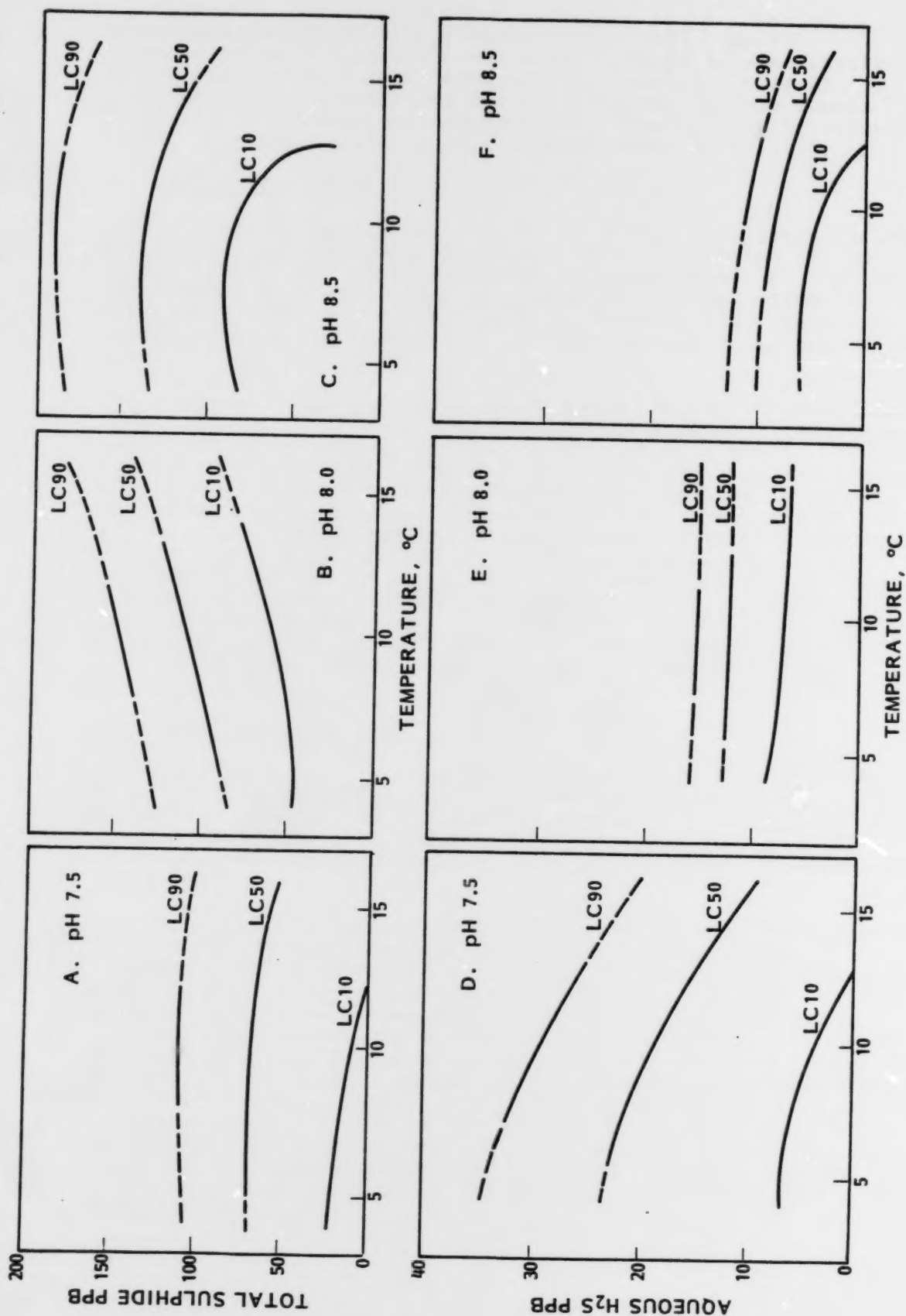


FIGURE 16
LAKE WHITEFISH SAC-FRY
H₂S BIOASSAY - 96 HOUR

those observed at pH 8.5 (Figures 15D to 15F). In all juvenile rainbow tests, a consistent increase in sensitivity to H_2S was observed with increased temperature. Thermal components were significant for all response surface models in Figure 15 (Appendices 10B to 10G). Generally, LC50 values at 5°C were approximately double those observed at 15°C.

C. Lake Whitefish Sac-Fry

Lake whitefish sac-fry were tested in 96 hour bioassays during the spring of 1979, immediately following completion of the 1 hour tests. Fry were acclimated to experimental pH and temperatures for at least 72 hours prior to testing. Experimental conditions and results are listed in Appendix 11A. Response surface models and Analyses of Variance are shown in Appendices 11B to 11G.

The response surface contour plots again indicate that increased levels of total sulphide were required to produce a given mortality as pH increased from 7.5 to 8.5 (Figures 16A to 16C). Figures 16D to 16F indicate that over this pH range, sensitivity to a given level of aqueous H_2S increased, as LC50 levels at pH 8.5 were less than half those at pH 7.5. While a significant increase in sensitivity to H_2S with increased temperature was observed in the pH 7.5 and pH 8.5 tests (Figures 16D and 16F, Appendices 11E and 11G), thermal effects were not significant for the pH 8.0 test (Figure 16E, Appendix 11F). Although the contour plot for the total sulphide model at pH 8.0 indicates a decreased sensitivity with increased temperature (Figure 16B) thermal effects were again not significant for this model (Appendix 11C).

D. Round Whitefish Sac-Fry

The round whitefish 96 hour sac-fry tests were done concurrently with the lake whitefish sac-fry tests during the spring of 1979. Acclimation conditions for the two species were similar. Test conditions and results are listed in Appendix 12A. Response surface models and Analyses of Variance are shown in Appendices 12B to 12G.

The contour plots indicate that in general, higher levels of total sulphide were required to produce a given mortality as pH increased, while increased sensitivity to aqueous H_2S was apparent over the pH 7.5 to pH 8.5 range (Figure 17). However, rather unusual results were obtained in the pH 8.0 and pH 8.5 tests. In both these tests, control (0 ppb H_2S) mortalities in excess of 10% were obtained at temperatures above 6°C. In the pH 8.0 test, control mortalities in excess of 50% were indicated for temperatures above approximately 14°C, while at pH 8.5, control mortalities greater than 50% were indicated for temperatures above 11°C. As shown in Figures 17A and 17D, high control mortalities were not obtained at these temperatures in the pH 7.5 test, and the 1 hour tests were characterized by low control mortalities at all pH and temperature levels.

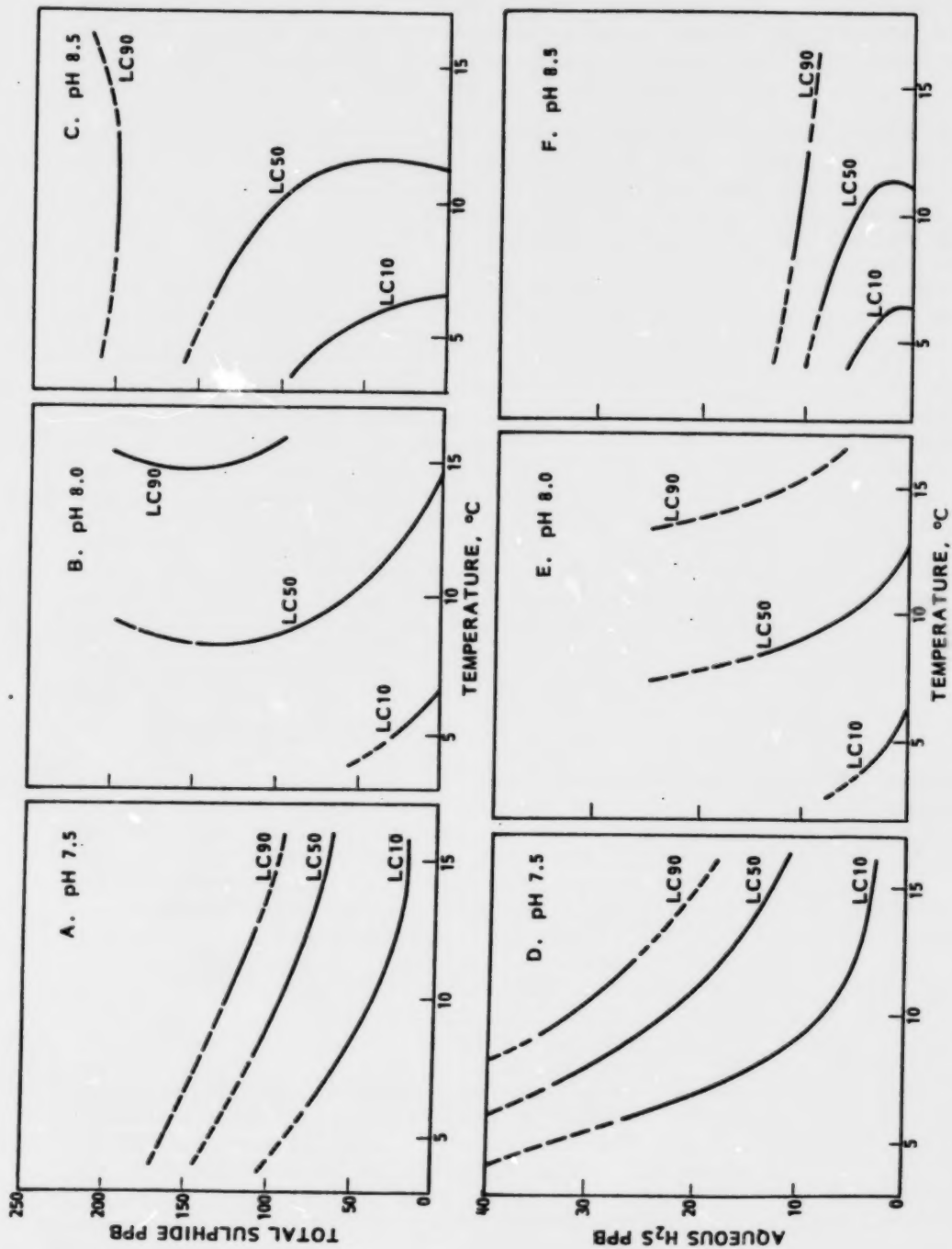


FIGURE 17
ROUND WHITEFISH SAC-FRY
H₂S BIOASSAY - 96 HOUR

A probable explanation for the control mortalities evident in Figure 17 lies in the sequential nature of the tests, combined with the fact that three 96 hour bioassays were necessary to establish toxic H_2S levels at pH 7.5. As the 1 hour bioassays were conducted first (over a two week period), followed by the pH 7.5 through pH 8.5 ninety-six hour tests, approximately seven weeks elapsed from the first 1 hour (pH 7.5) test to completion of the last (pH 8.5) 96 hour test.

Lake whitefish were tested concurrently with the round whitefish. As the lake whitefish hatched over a period in excess of 40 days, sufficient numbers of newly hatched fry were available for testing of this species over the entire experimental period. Better control survival was evident for lake whitefish at warm temperatures, although some control mortality was observed for lake whitefish tested at the warmest temperatures in the 96 hour bioassays (Figure 16). As the round whitefish hatched more rapidly (hatch duration was somewhat less than 30 days), the last tests (96 hour pH 8.0 and pH 8.5) were conducted with fish which had hatched some time (up to two weeks) prior to testing. These fish showed high control survival at cold temperatures, but a progressive increase in control mortality with increased temperature and time (compare pH 8.0 and pH 8.5 tests). These results suggest that at warm temperatures, insufficient yolk-sac reserves were present for survival of the 72 hour acclimation periods, followed by the 96 hour test periods. Hence, it appears that the round whitefish 96 hour test results at the high pH levels (8.0, 8.5) should be discarded. Only the results obtained at pH 7.5 should be considered as reliable.

DISCUSSION

1. Comparison With MacLaren's Study

One of the objectives of the present study was the comparison of results with those obtained by MacLaren (1977). As MacLaren's tests were not replicated, a direct statistical comparison cannot be readily made. However, it was felt that results of the two studies might be best compared by consideration of both the calculated LC50 values and the ranges over which mortality occurred. As previously indicated, the LC10 to LC90 contours of the response surface models appear to provide a good indication of the range of H_2S levels causing mortality for the results of the present study. From original data sheets included in MacLaren's report, some indication of the range of H_2S values causing mortality can also be obtained. Where possible, H_2S concentrations causing low mortality levels (<10%) and those causing high mortality levels (80% to 100%) have been abstracted from MacLaren's data. These and the calculated LC50 values have been superimposed on contour plots (LC10 to LC90) from the present study. From such comparisons, it is possible to determine if substantial differences exist between results of the two studies.

A. Rainbow Trout Sac-Fry

In MacLaren's study, rainbow trout sac-fry were used in 1 hour bioassays at 7°C and pH 7.5, at 7°C and pH 8.0, and 10°C and pH 8.0. As indicated in Figure 18, present results differ from

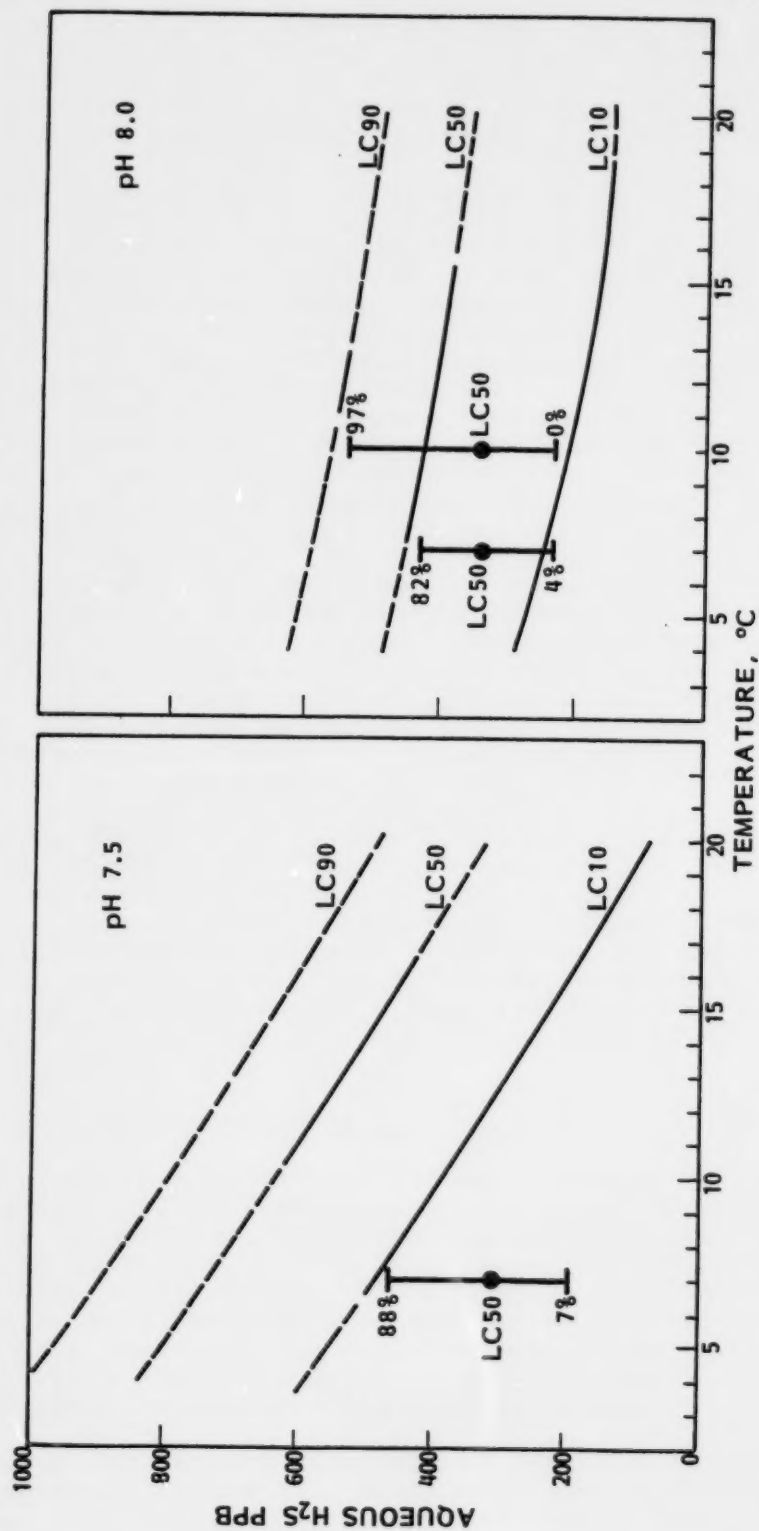


FIGURE 18
COMPARISON OF PRESENT RESULTS WITH THOSE OF MACLAREN (1977)
1 HOUR BIOASSAY ON RAINBOW TROUT SAC-FRY

MacLaren's in the pH 7.5 tests. Present results indicate that at pH 7.5 and 7°C, mortality would be expected over the 500 ppb to 900 ppb aqueous H₂S range, with the LC50 occurring at slightly above 700 ppb. In contrast, MacLaren obtained 7% mortality at 200 ppb, 88% mortality at 450 ppb, and a calculated LC50 value of 300 ppb. Clearly, the results of the two studies are substantially different for these test conditions.

Better correspondence was obtained at pH 8.0 (Figure 18). At 10°C, MacLaren's LC50 was slightly lower (350 ppb) than that indicated by the present study (425 ppb) and the range over which mortality occurred (0% to 97%) was somewhat narrower than that of the present study. However, the results are quite similar, given the variability inherent in such data. At 7°C and pH 8.0, MacLaren's results indicated a substantially lower LC50 than that of the present study, and a much smaller range of H₂S concentrations between first mortality (4%) and almost complete mortality. As shown in Figure 18, MacLaren obtained 82% mortality at H₂S levels below the LC50 value of the present study. While these results must be considered substantially different, the degree of correspondence is still better than that obtained at pH 7.5. In Figure 18, MacLaren's results have been compared with the Group 2 rainbow sac-fry of the present study. Results for the Group 1 rainbow sac-fry were very similar for the pH 7.5 test. The results were also similar at warm temperatures and pH 8.0, but the Group 1 fish showed better survival at low test temperatures. Hence, if comparisons were made between MacLaren's results and those of the Group 1 sac-fry, greater differences would be apparent for the 7°C and pH 8.0 test condition.

Results for 96 hour rainbow trout sac-fry bioassays are compared in Figure 19. MacLaren's results for the pH 7.5 and 7°C test condition indicate a slightly greater sensitivity to H₂S than results of the present study. However, the differences are relatively minor. Greater differences are apparent for the pH 8.0 tests at 10°C. In this case, MacLaren's rainbow trout sac-fry were substantially more resistant, as their LC50 value occurred at H₂S levels close to the LC90 of the present study (Figure 19).

B. Rainbow Trout Juveniles

MacLaren (1977) conducted 1 hour bioassays on rainbow trout juveniles under assumed winter (pH 7.5, 7°C) and summer (pH 8.0, 20°C) conditions. These results are compared with present models in Figure 20. In both tests, MacLaren's results indicated much lower LC50 values than those obtained in the present study. Also, the range of H₂S values between 0% mortality and complete mortality was much narrower in MacLaren's study. In both tests, MacLaren obtained complete mortality at H₂S levels less than the LC10 for the present study.

Results for 96 hour bioassays on juvenile rainbow trout are compared in Figure 21. Again, MacLaren's results indicated much lower LC50 values than those obtained in this study, and a narrower range of H₂S levels between complete survival and complete mortality.

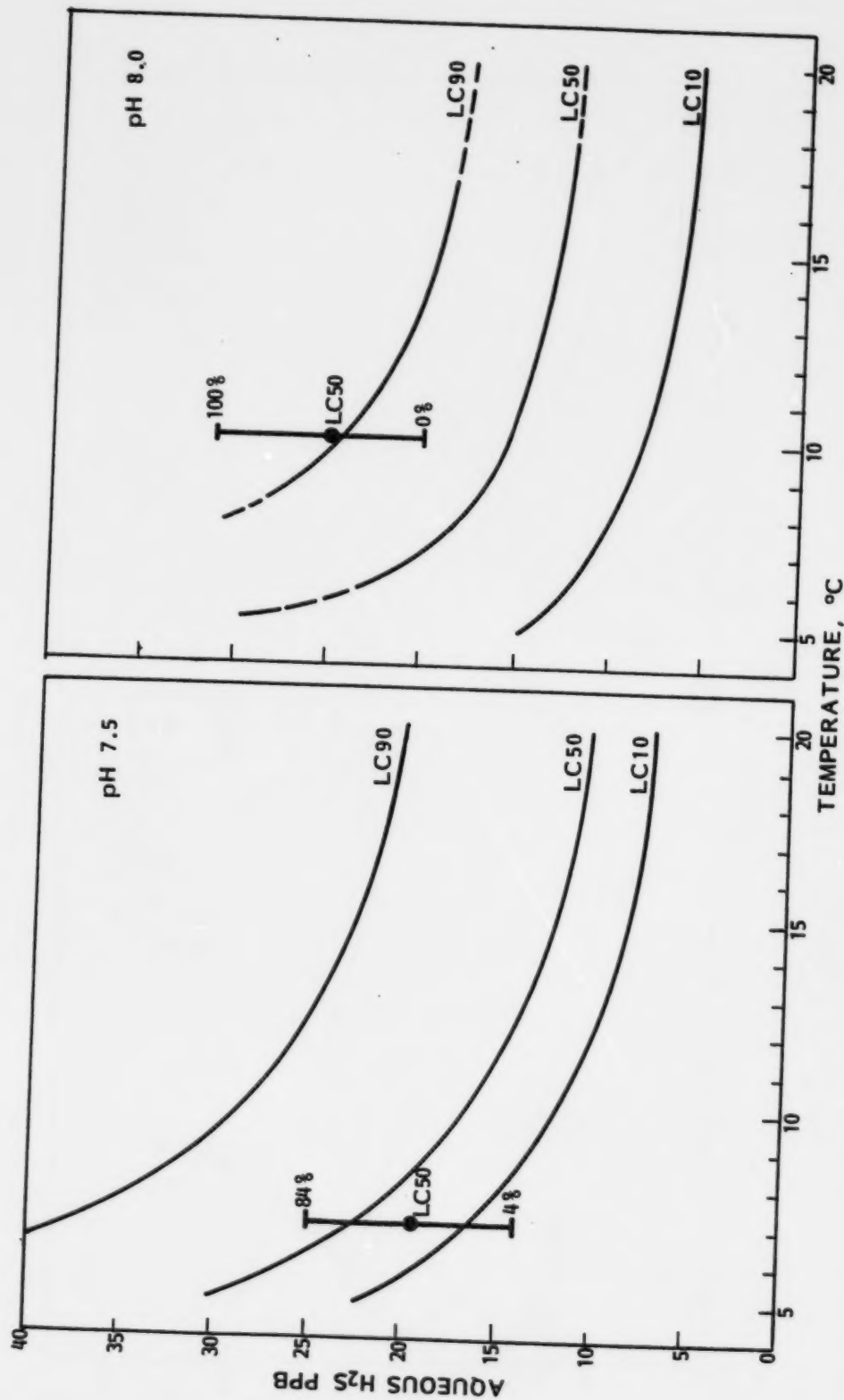


FIGURE 19
COMPARISON OF PRESENT RESULTS WITH THOSE OF MACLAREN (1977).
96 HOUR BIOASSAY ON RAINBOW TROUT SAC-FRY

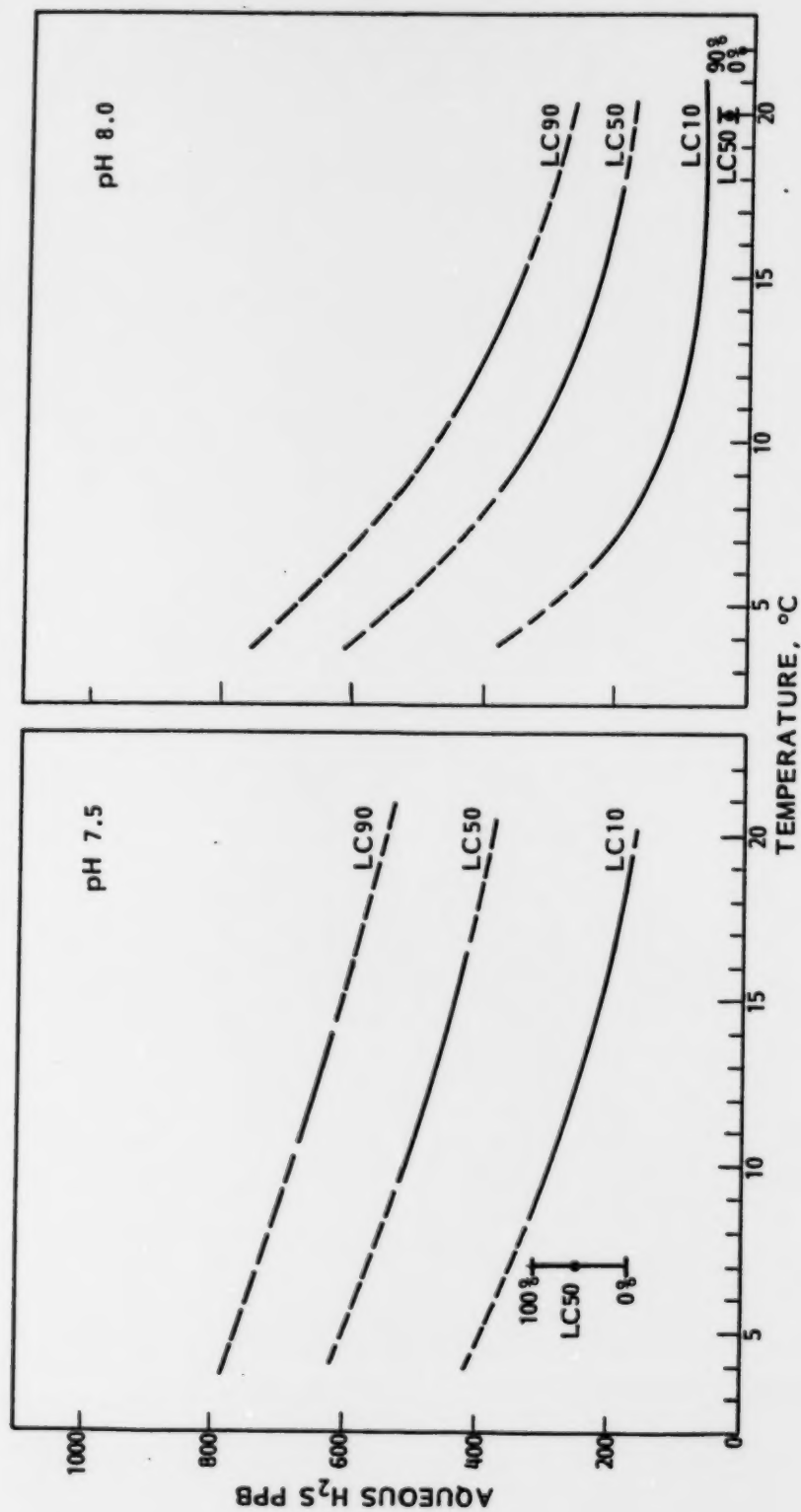


FIGURE 20
COMPARISON OF PRESENT RESULTS WITH THOSE OF MACLAREN (1977)
1 HOUR BIOASSAY ON RAINBOW TROUT JUVENILES

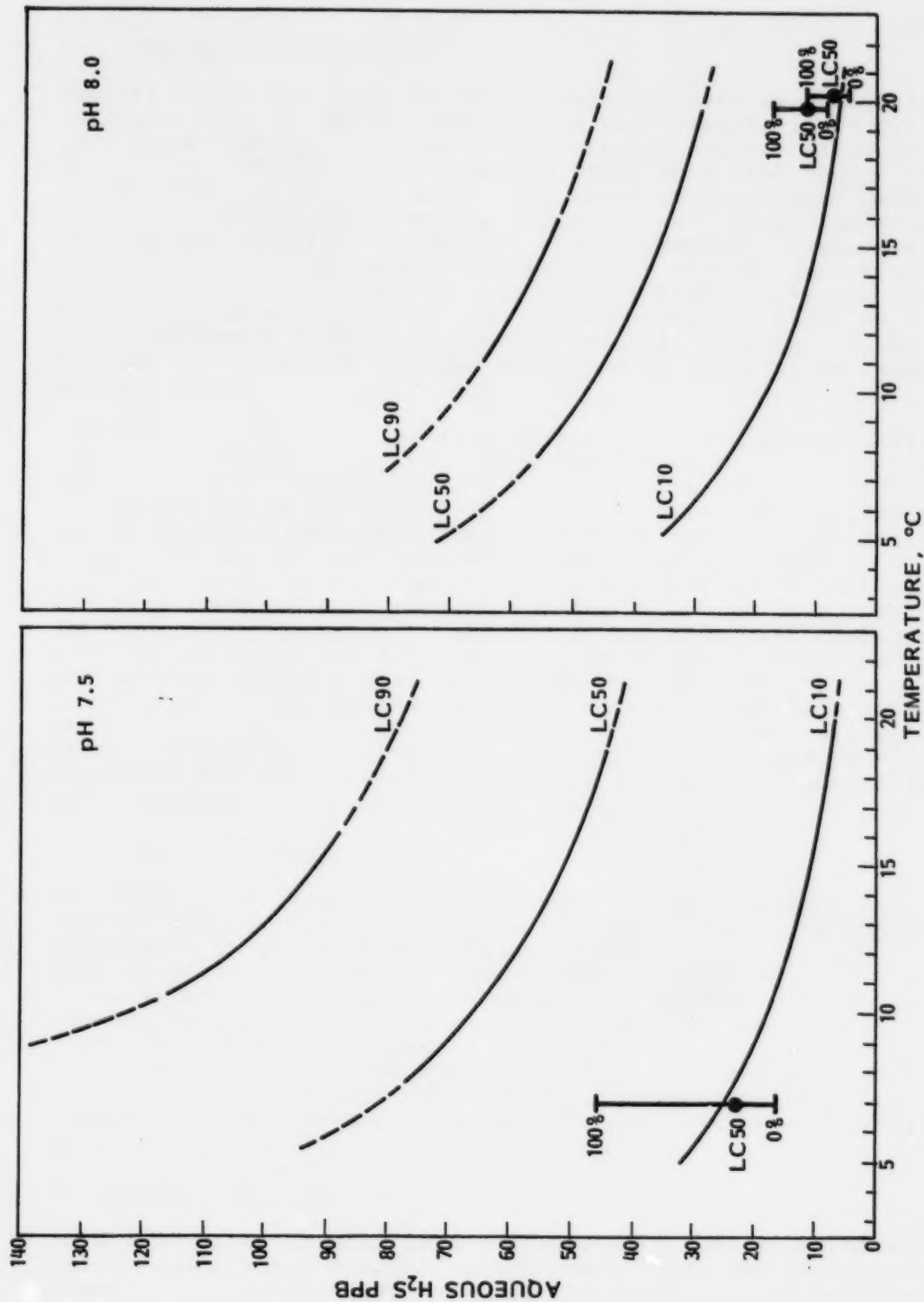


FIGURE 21
COMPARISON OF PRESENT RESULTS WITH THOSE OF MACLAREN (1977).
96 HOUR BIOASSAY ON RAINBOW TROUT JUVENILES

C. Lake Whitefish Sac-Fry

Results for 1 hour lake whitefish sac-fry tests are compared in Figure 22. At pH 7.5, MacLaren's test results (at 7°C) were quite similar to those of the present study. Although MacLaren's LC50 value was somewhat lower than that indicated by the response surface model, the range of H₂S values between complete survival and complete mortality was almost identical for the two studies. At pH 8.0, both of MacLaren's sac-fry tests (7°C, 10°C) indicated substantially greater sensitivity to H₂S than the response surface model fitted to present results. MacLaren's results for juvenile lake whitefish at 10°C were comparable to present sac-fry results, although slightly greater resistance to H₂S was suggested by the sac-fry response surface model.

Comparison of results obtained in the 96 hour lake whitefish sac-fry tests indicates that much greater sensitivity to H₂S was observed in all MacLaren's tests (Figure 23). In all but one of the tests (pH 8.0, 10°C) MacLaren obtained complete mortality at H₂S levels below the LC10 contours of the present study. Of the 96 hour lake whitefish tests conducted by MacLaren, only the results for juveniles (not shown in Figure 23) were comparable to present sac-fry data. In this test (pH 8.0, 10°C) MacLaren's LC50 was 12 ppb H₂S, with complete survival at 5 ppb and 80% mortality at 14 ppb. Thus, the comparisons indicate that in all but one (pH 7.5, 7°C) of the sac-fry tests, MacLaren's whitefish were substantially more sensitive to H₂S than sac-fry tested in the present study. Present whitefish sac-fry results in both 1 hour and 96 hour bioassays are generally comparable to the juvenile whitefish data obtained by MacLaren.

D. Rock Bass Sac-Fry

MacLaren (1977) conducted 1 hour and 96 hour bioassays on another centrarchid species, the largemouth bass. In Figure 24, MacLaren's 1 hour test results are compared with the 1 hour rock bass sac-fry models of the present study. At pH 7.5 and 15°C, MacLaren's largemouth bass sac-fry displayed slightly greater sensitivity to H₂S than the rock bass. However, largemouth juveniles were somewhat more resistant. At pH 8.0 and 20°C, MacLaren's largemouth sac-fry were substantially more sensitive than the rock bass sac-fry. Present results are very similar to those obtained by MacLaren for largemouth juveniles at pH 8.0 and 25°C.

E. Summary of Comparisons

It was possible to make direct comparisons between MacLaren's (1977) results and those of the present study for 18 bioassay tests. In only one test (rainbow trout sac-fry, 96 hour bioassay at pH 8.0 and 10°C) did MacLaren's LC50 substantially exceed the value indicated by the present results. In two tests, results of both studies were nearly identical, while in six

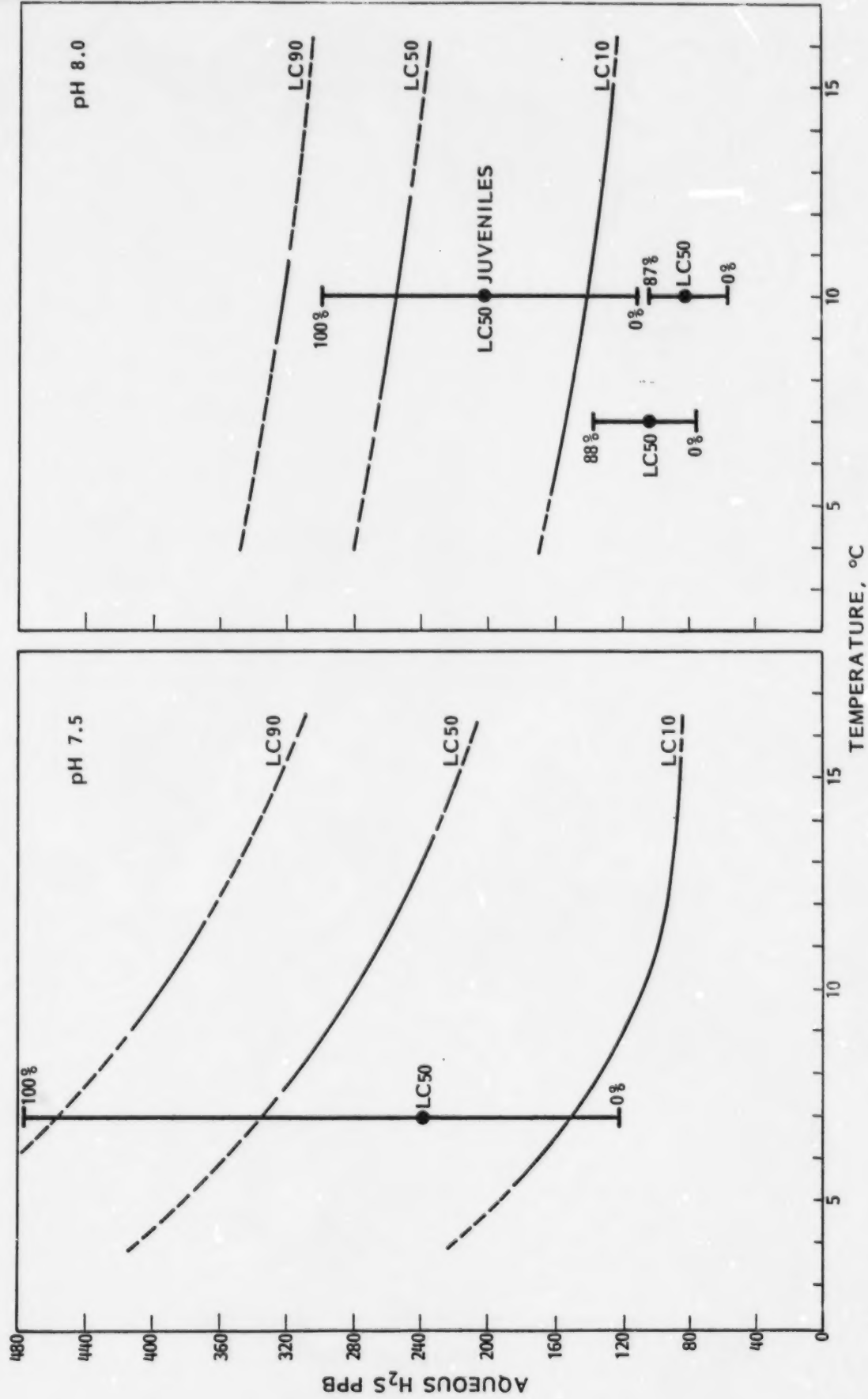


FIGURE 22

COMPARISON OF PRESENT RESULTS WITH THOSE OF MACLAREN (1977)
1 HOUR BIOASSAY ON LAKE WHITEFISH SAC-FRY

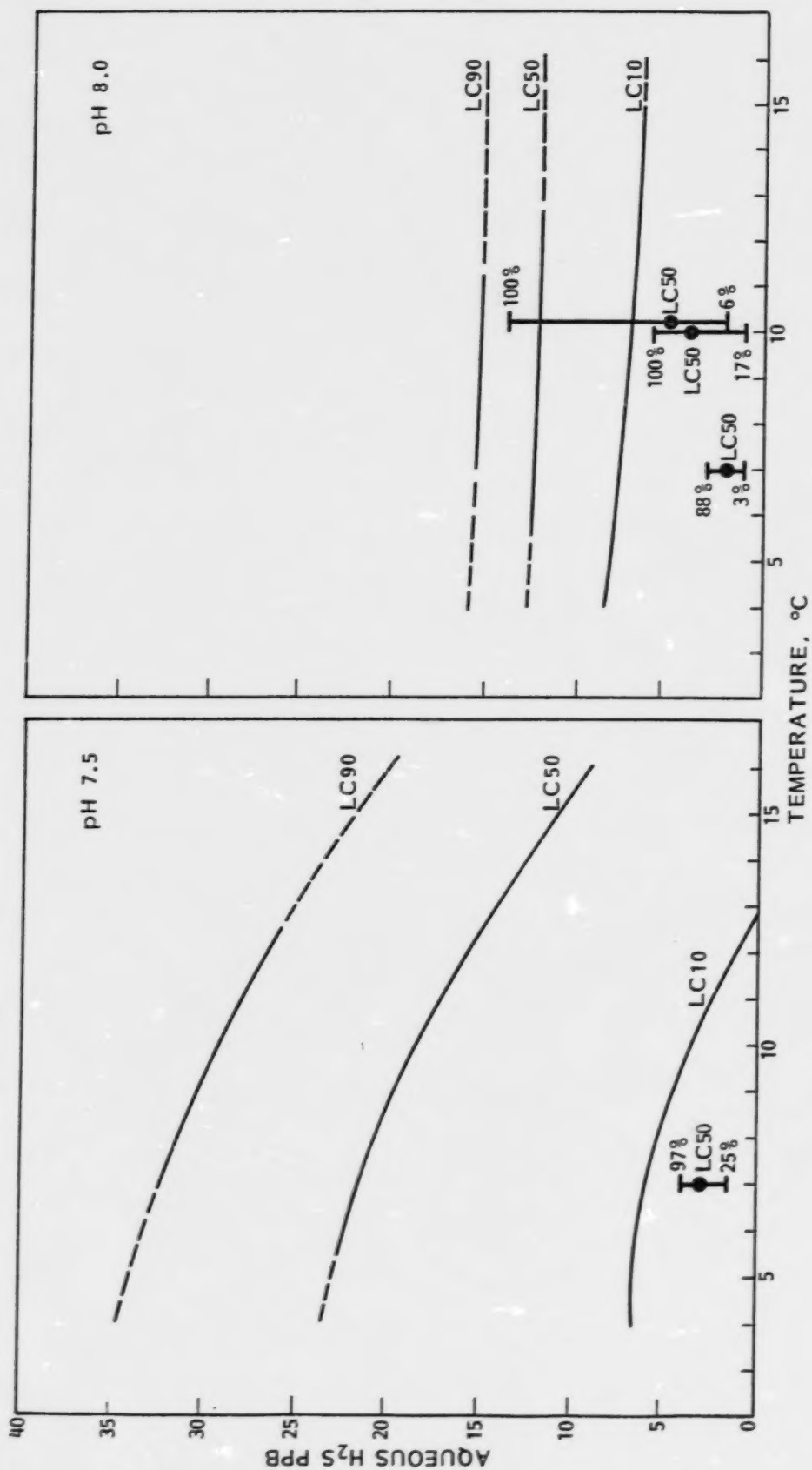


FIGURE 23
COMPARISON OF PRESENT RESULTS WITH THOSE OF MACLAREN (1977)
96 HOUR BIOASSAY ON LAKE WHITEFISH SAC-FRY

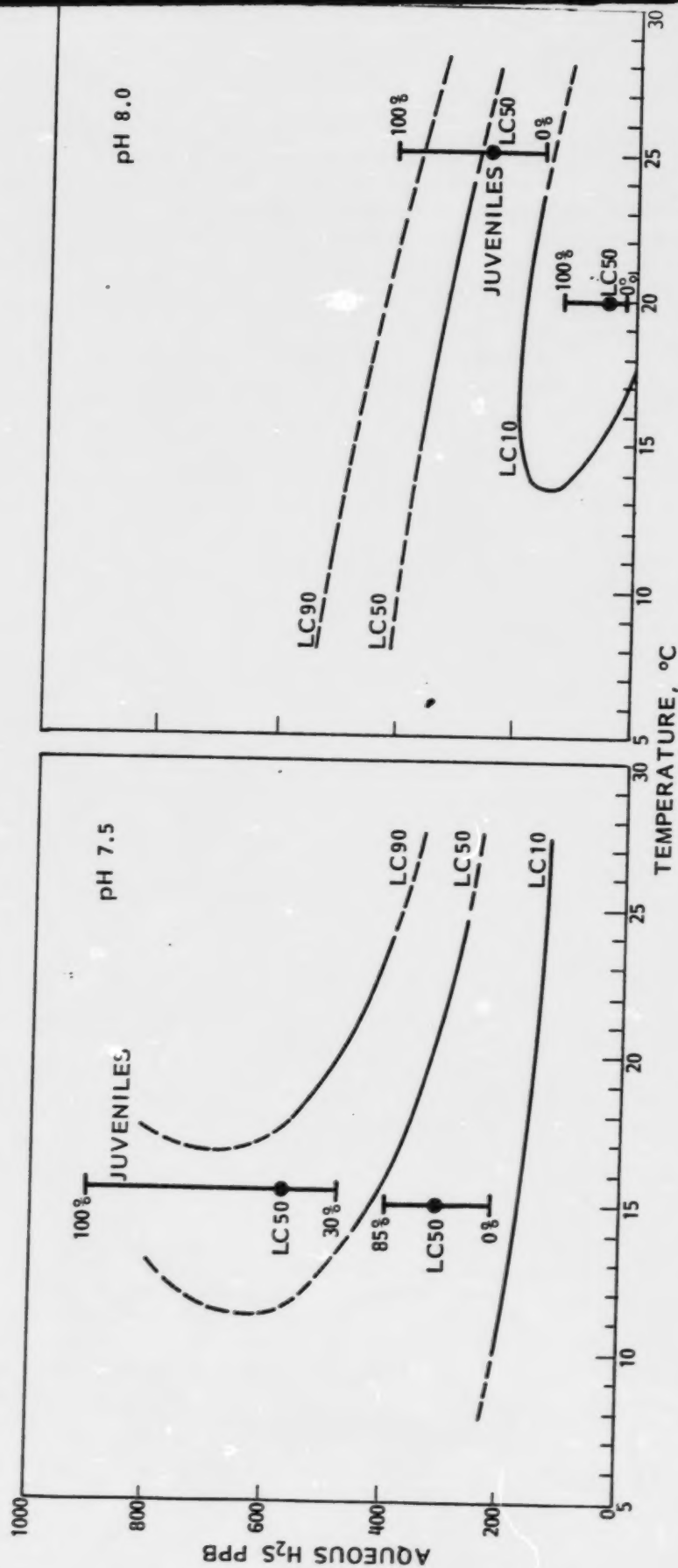


FIGURE 24
COMPARISON OF PRESENT RESULTS WITH THOSE OF MACLAREN (1977).
1 HOUR BIOASSAY WITH ROCK BASS SAC-FRY AND LARGEMOUTH BASS SAC-FRY (MACLAREN)

experiments, MacLaren's LC50 values were somewhat lower than present values (MacLaren's LC50 value occurred at H₂S levels below present values, but above the LC10 of present tests). In the nine other cases, MacLaren's results were much lower than present values. In these nine tests, MacLaren's LC50 values occurred at H₂S levels below the LC10 of the response surface models, and in most cases, total mortality was obtained at H₂S concentrations below the LC10 of the present study.

In general, it is apparent that MacLaren's results indicate greater sensitivity to H₂S than was found in the present study. Where results for both sac-fry and juveniles were available, present sac-fry results appeared more comparable to MacLaren's juvenile data. In those cases where substantial differences in results exist, there is no clear reason for declaring a particular data set in error. Differences in fish stocks used in bioassays may contribute to variability in results, as was observed for the two rainbow trout sac-fry 1 hour bioassays of the present study. Also, differences in bioassay techniques or H₂S analysis procedures may have influenced observed results. The health of test fish may also have contributed to differences in results, especially for rather fragile sac-fry stages with limited yolk reserves. In any cases where the health of test fish was sub-optimal, lower tolerance to toxic substances would be expected. Also, it should be noted that each bioassay in MacLaren's study consisted of six tests (five levels of H₂S plus controls). Response surface models of the present study were based on a minimum of 33 test values. In some cases, where bioassays were repeated due to minimal mortality in the initial tests, as many as 99 data values were used to define a single response surface model. Hence, models of the present study are based on substantially more data than the LC50 values reported by MacLaren.

2. Effects of Temperature and pH on H₂S Toxicity

A. Temperature Effects on Toxicity

Models of the present study have generally indicated a statistically significant decrease in tolerance to hydrogen sulphide as experimental temperatures increased. As the dissociation of H₂S is increased with temperature, the proportion of the most toxic, aqueous form decreases somewhat as temperature increases. Thus, thermal effects are most clearly defined for the aqueous H₂S models. Smith et al (1976) presented data which indicated substantial declines in the H₂S aqueous LC50 for fathead minnow, goldfish and brook trout. Results for the first two species indicated linear declines in the LC50 as temperature increased when data were log-transformed. However, results for brook trout differed, as the LC50 declined over the 8°C to 16°C range, then rose slightly as test temperatures approached 20°C.

If the toxicity of aqueous H₂S is primarily a function of the rate at which the H₂S molecules are taken up through the fish gill, then toxicity should be directly correlated to the rate of gill ventilation, and hence metabolism. A large volume of

literature exists regarding the effects of temperature on fish metabolism (see reviews by Fry, 1947, 1964, 1967; Hochachka and Hayes, 1962; Winberg, 1956). In those cases where the confounding effects of spontaneous activity can be adequately removed, ie, where true basal or standard metabolic rate has been measured, the majority of studies indicate a Q_{10} value from 1.8 to 2.0 for acclimated fish (Evans, MS 1977; Griffiths, MS 1978). Thus, over a 10°C span of temperatures, (provided that these temperatures are not near lethal limits) one would expect the standard metabolic rate, and hence, the ventilation rate, to approximately double. If toxicity of aqueous H_2S is primarily dependent upon the rate of uptake through the gill, then it might also be expected that the LC50 would decline by 50% as the result of a 10°C temperature rise.

Present data have indicated a considerable variability in the effect of temperature on H_2S toxicity. In order to obtain a generalized relationship between temperature and H_2S toxicity, results were pooled across the various pH levels and species tested. Three tests which produced apparently aberrant results (1 hour rock bass sac-fry at pH 8.5; 96 hour round whitefish sac-fry at pH 8.0 and pH 8.5) were deleted from this analysis. The reasons for considering these results unreliable have been outlined in the Results section of this report. In order to eliminate between-test differences in the absolute levels of H_2S tolerated, the effect of temperature was considered in relation to a low-temperature baseline value (ie, the aqueous LC50 at 5°C) which was assigned a value of 100%. From the response surface models the percentage decline in the LC50 value was calculated for temperature increases of 5°C and 10°C above the baseline (cold temperature) value. Results were tabulated separately for the 1 hour and 96 hour tests. Regressions were fitted relating the proportional decline ($\log_{10}$ of baseline percentage) and the increase in temperature. Results are shown for the 1 hour tests (20 experiments, $n = 60$) and the 96 hour tests (10 experiments, $n = 30$) in Figure 25. While it is apparent that substantial variation exists in the data, an overall significant decline in LC50 values resulted from increased test temperatures. Regression slopes for both 1 hour and 96 hour tests were very similar. Thus, Q_{10} values for both tests were slightly below 2, indicating that the general effect of a 10°C temperature increase would be to decrease the LC50 by slightly less than 50%.

Results of the present study have supported the contention that toxicity of aqueous H_2S is the result of uptake rates through the fish gill, as the increased sensitivity resulting from increased temperature is very similar to the expected metabolic rate increase. However, due to high variability in the present data (and the lack of direct metabolic measurements in the present bioassays) this conclusion should remain tentative until supported by further studies.

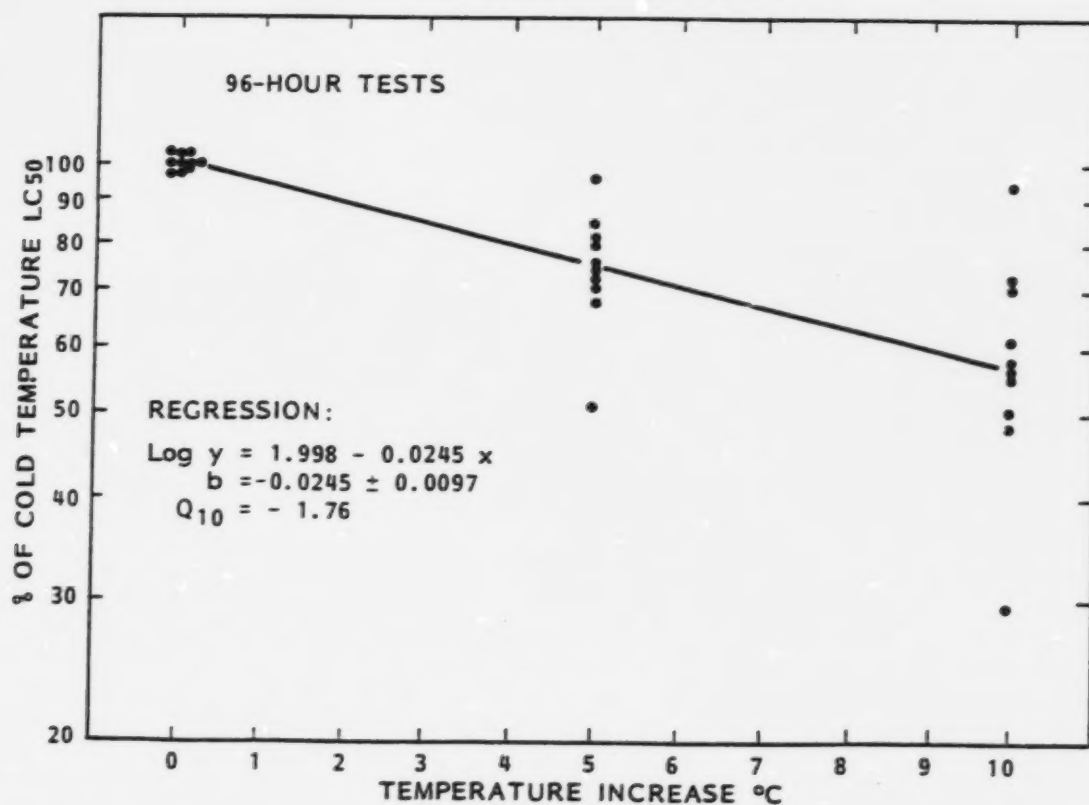
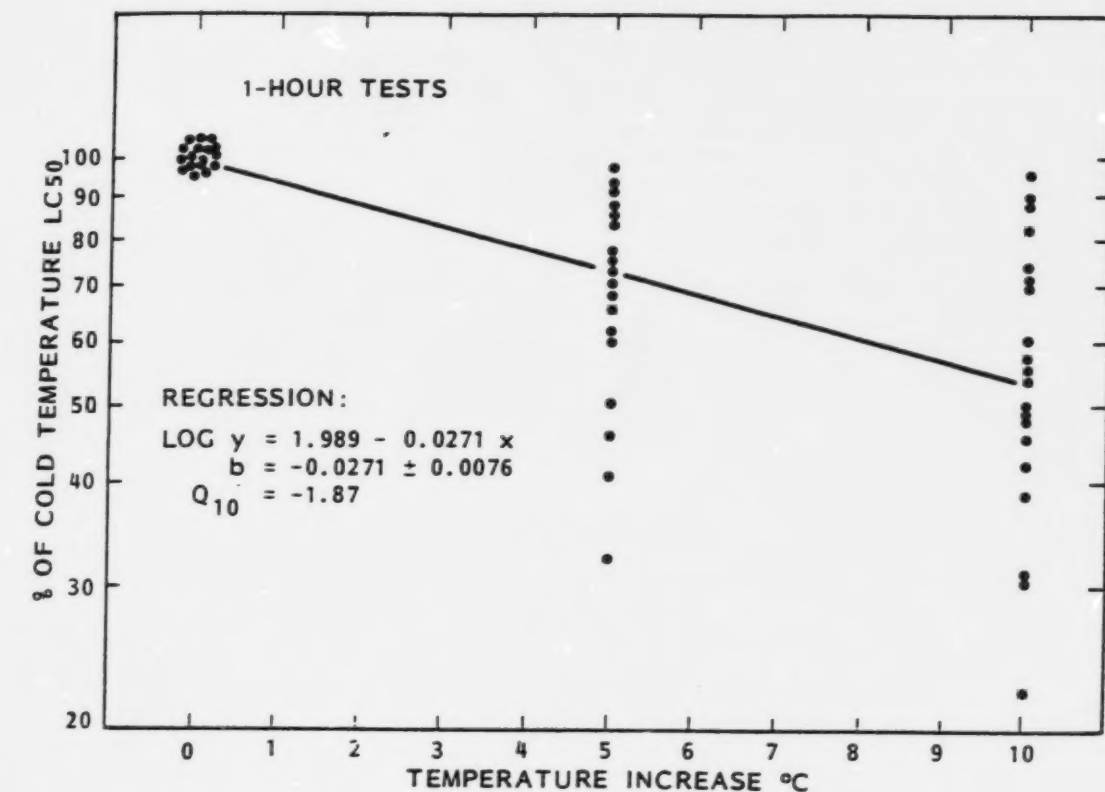


FIGURE 25
EFFECT OF INCREASED TEMPERATURE ON
AQUEOUS LC50 VALUES IN 1-HOUR AND 96-HOUR TESTS
DATA ABSTRACTED FROM RESPONSE SURFACE MODELS

B. pH Effects on Toxicity

Comparison of the response surface models fitted for each experimental pH in the present study has indicated that in general, toxicity of a given level of aqueous H_2S increased over the pH 7.5 to pH 8.5 range. While the H_2S (aqueous) LC50 thus declined over this pH range, considerable variability was evident in the results from the various test series conducted. In order to obtain a generalized assessment of pH effects on the response to aqueous H_2S , a procedure similar to that used in the previous assessment of thermal effects was adopted. In the present case, comparisons were made at a constant temperature for a series of pH levels, generally the temperature at the centre of the experimental design, where the response surface models were considered most reliable.

The LC50_(aq) value at the lowest pH (7.5) was assigned a value of 100% in each test series. From the contour plots, the LC50 values at pH levels of 8.0 and 8.5 were abstracted and converted to percentages of the pH 7.5 value. Then the percentage values (log scale) were regressed against the pH levels (mean observed for each experiment) to obtain a generalized relationship between pH and aqueous H_2S toxicity. This procedure eliminated differences in absolute levels of H_2S tolerated by various fish species or life stages, yet retained the inherent variability in the toxicity/pH relationships. As with the thermal analysis, results were considered separately for the 1 hour and 96 hour tests.

As indicated by the regressions in Figure 26, significant decreases in the LC50 values occurred in both the 1 hour and 96 hour tests. While a somewhat greater regression slope was observed for the 96 hour tests, variability in the data was sufficient to prevent the two regression slopes from being declared significantly different (see 95% confidence intervals of the slopes in Figure 26). Thus, it may be generally concluded that over the pH 7.5 to pH 8.5 range, the aqueous H_2S LC50 will decline by approximately 60%. In Figure 26, results are also shown for the 96 hour fathead minnow tests conducted by Smith et al (1976). Again, results have been considered in terms of percentage decline from the pH 7.5 value. It is apparent that the fathead minnow results agree closely with those of the present study.

The results in Figure 26 indicate that either the HS^- ion contributes to the toxicity of a hydrogen sulphide solution, or that fish are more sensitive to a given H_2S _(aq) level over the pH 7.5 to pH 8.5 range. As this range of pH values is well within the zone considered optimal for fish, it would seem likely that a low level HS^- toxicity is the cause of the observed results, especially when the substantial increase in HS^- concentration (at a fixed H_2S _(aq) level) is considered. Smith et al (1976) supported the contention that the HS^- ion contributes to the observed toxicity. They pointed out that within the body of the fish, sulphide species (including HS^-) inhibit

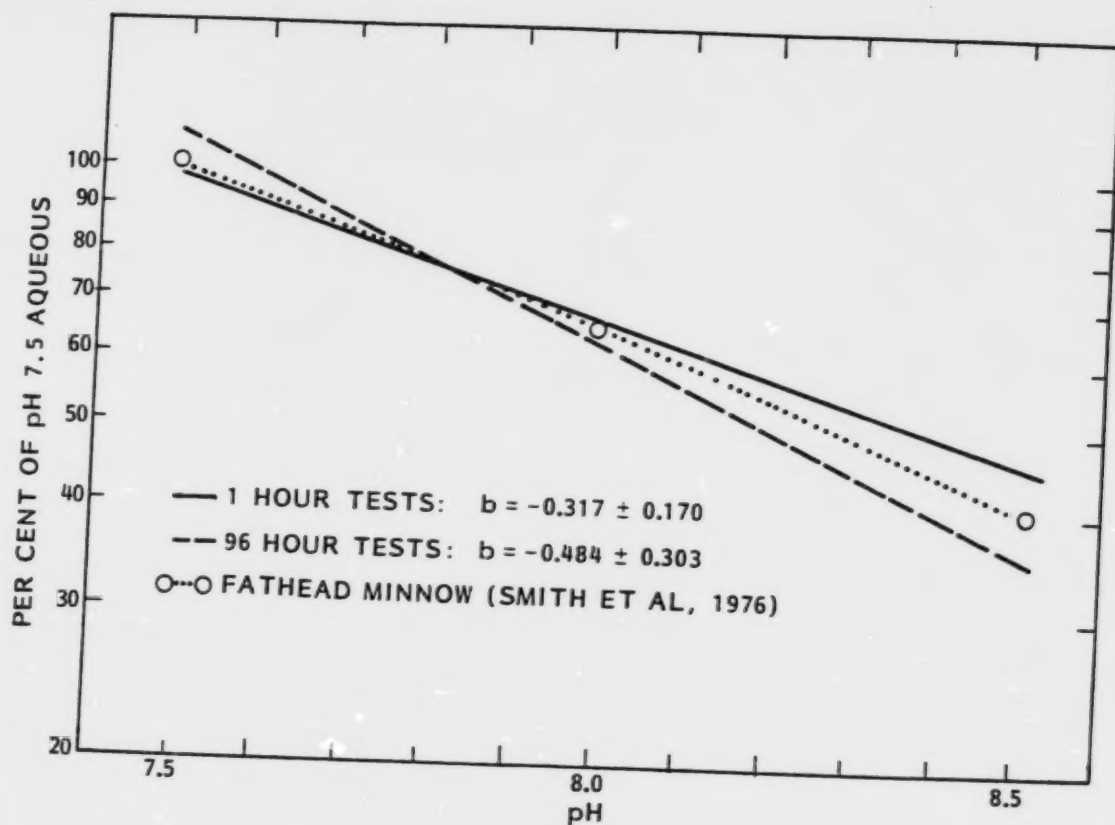


FIGURE 26
DECLINE IN AQUEOUS H_2S LC50 WITH EXPERIMENTAL pH

enzymes by formation of complexes with essential metal ions in enzymes such as peroxidase and catalase (White et al, 1968). Sulphide species also bind to the ferric ion of cytochrome oxidase and thus inhibit oxygen metabolism. Smith et al (1976) thus suggest that the lower toxicity of HS^- (compared with aqueous H_2S) is due to the reduced ability of the charged ion to pass through the gills rather than substantial differences in toxicity once the ion has entered the body of the fish. However, given the high concentration of HS^- ions present at high pH levels, a measurable toxic effect is evident.

As the proportions of aqueous H_2S and HS^- ions present can be readily calculated for each pH level, it is possible to estimate the relative toxicity of these components of a hydrogen sulphide solution. For the purposes of this estimate, a pooled regression slope (-0.4005) has been obtained from the 1 hour and 96 hour tests. At pH 7.5, if it is assumed that 50% mortality will occur at an aqueous H_2S level of 100 units (actual concentration may be ignored), then equivalent mortality will occur from 39.8 units of aqueous H_2S at pH 8.5. This level of 100 units aqueous H_2S will be accompanied by 235.6 units of HS^- (Clarke, 1974) at an assumed temperature of 15°C. At pH 8.5, the 39.8 units of aqueous H_2S will be accompanied by 938.1 units of HS^- . Since these two solutions of H_2S will produce equivalent toxicity (LC50), then they may be equated:

$$100 \text{ H}_2\text{S}_{(\text{aq})} + 235.6 \text{ HS}^- = 39.8 \text{ H}_2\text{S}_{(\text{aq})} + 938.1 \text{ HS}^-$$

This may be simplified by subtraction:

$$60.2 \text{ H}_2\text{S}_{(\text{aq})} = 702.5 \text{ HS}^-$$

or:

$$1 \text{ H}_2\text{S}_{(\text{aq})} = 11.67 \text{ HS}^-$$

Thus, assuming that the response of fish to $\text{H}_2\text{S}_{(\text{aq})}$ is unchanged over the pH 7.5 to pH 8.5 range, and that the observed regressions in Figure 26 are due to the presence of varying amounts of slightly toxic HS^- ions present, the above calculations suggest that the HS^- ions in the water have a toxic effect about 8% (ie, one-twelfth) of that displayed by aqueous H_2S molecules. Smith et al (1976) found that the 96 hour LC50 values for fat-head minnows declined from 57.3 ppb $\text{H}_2\text{S}_{(\text{aq})}$ at pH 7.1 to 14.9 ppb $\text{H}_2\text{S}_{(\text{aq})}$ at pH 8.7 (only their pH 7.5 to pH 8.5 data are shown in Figure 26). If the methodology employed above is used for the data of Smith et al (1976), the calculations indicate that the HS^- ion is approximately one-fourteenth as toxic as aqueous H_2S . If the hydrosulphide ion has similar toxicity to the H_2S molecule once it has entered the body of a fish (as implied by Smith et al, 1976), then the ratio of toxicities suggested by the above calculations may be indicative of the rate-limiting step of the toxic process, ie, the relative rates at which H_2S molecules and HS^- ions pass through the fish gill.

3. Comparison with Proposed Emission Limits for Bruce Heavy Water Plants

Guest (1978) proposed that emissions from the Bruce Heavy Water Plants should not exceed 175 ppb (total sulphide) averaged over 1 hour, 95 ppb averaged over 24 hours, or 20 ppb averaged over 1 year. The Ministry of the Environment reduced these limits to 105 ppb averaged over 1 hour and 82 ppb averaged over 24 hours. A long-term emission limit was not specified. As present studies have utilized 1 and 96 hour tests, both the MOE and Guest (1978) emission limits will be used for comparison with present results.

These criteria were based on the assumption that the most restrictive conditions would occur during the summer months. Results of the present study allow evaluation of these proposed emission limits on a seasonal basis, considering the temperatures and pH levels likely to prevail during the seasons that particular species or life stages might be present in the area of the BHWP discharge.

Of the species and life stages examined in the present study, only juvenile rainbow trout are likely to be exposed to the BHWP discharge at all seasons of the year. In Figure 27, rainbow juvenile toxicity results are compared with the proposed emission limits for each season. The expected pH levels and ambient temperatures for each season are indicated on the figure. The toxic H_2S levels at temperatures expected in the immediate discharge ($10^\circ \Delta T$) and in the far field ($2^\circ \Delta T$) were obtained from the appropriate response surface model. These are shown in the figure by the vertical bars, which represent the range of H_2S concentrations causing mortality (LC10 to LC90). The LC50 values are indicated by the circles contained within each bar. For the summer condition, a near-field ΔT of only $6^\circ C$ has been used. As the ultimate upper lethal temperature of rainbow trout approximates $25^\circ C$, and the final preferendum is in the $15^\circ C$ to $20^\circ C$ range, it seems unlikely that rainbow juveniles would remain in temperatures much greater than $23^\circ C$ (ambient $+6^\circ \Delta T$) for extended periods of time.

It is clear from Figure 27 that the proposed BHWP emission limits are substantially below the toxic H_2S levels determined in the present 1 hour and 96 hour bioassay tests. The apparent safety margin is substantial for all conditions. The lowest safety margin is for the 96 hour winter exposure (Figure 27A) in the immediate discharge (near-field) where the LC10 value is approximately double the proposed maximum emission limit. However, the assumed pH (7.5) is probably somewhat lower than the true winter pH at the BHWP site.

Results of the present study and those in the literature (Smith et al, 1976; Clarke, 1974; Guest, 1978) have generally indicated that the sac-fry of most species is the life stage most sensitive to H_2S . The periods when sac-fry are likely to be present in the BHWP area are quite brief. In Figure 28, toxicity data from the present study are compared with proposed emission limits for the periods when sac-fry of each species could be present in the BHWP area. Both lake whitefish and

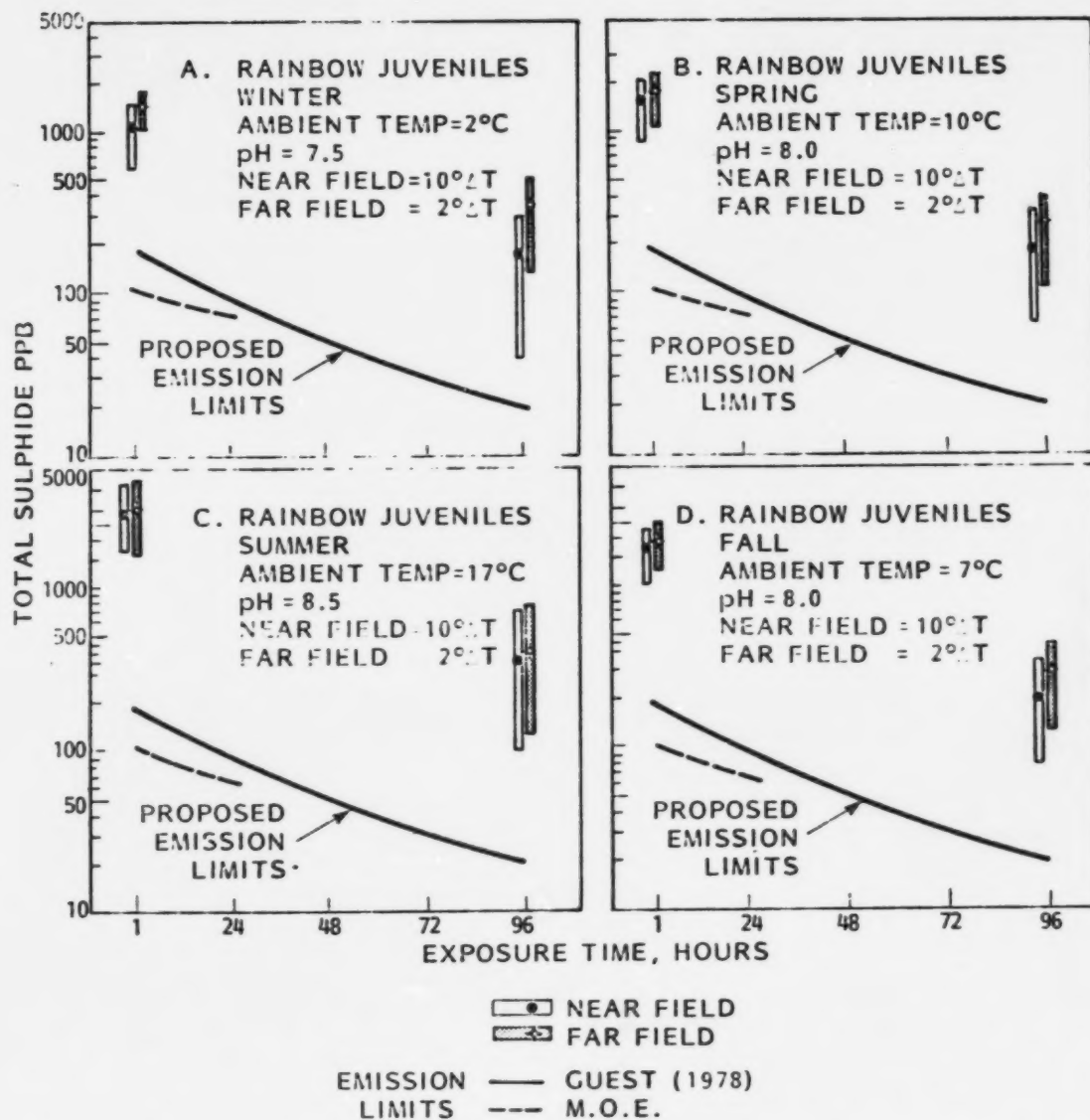


FIGURE 27

COMPARISON OF RAINBOW TROUT JUVENILE SEASONAL TOXICITY DATA
 WITH PROPOSED EMISSION LIMITS FOR BRUCE HEAVY WATER PLANT

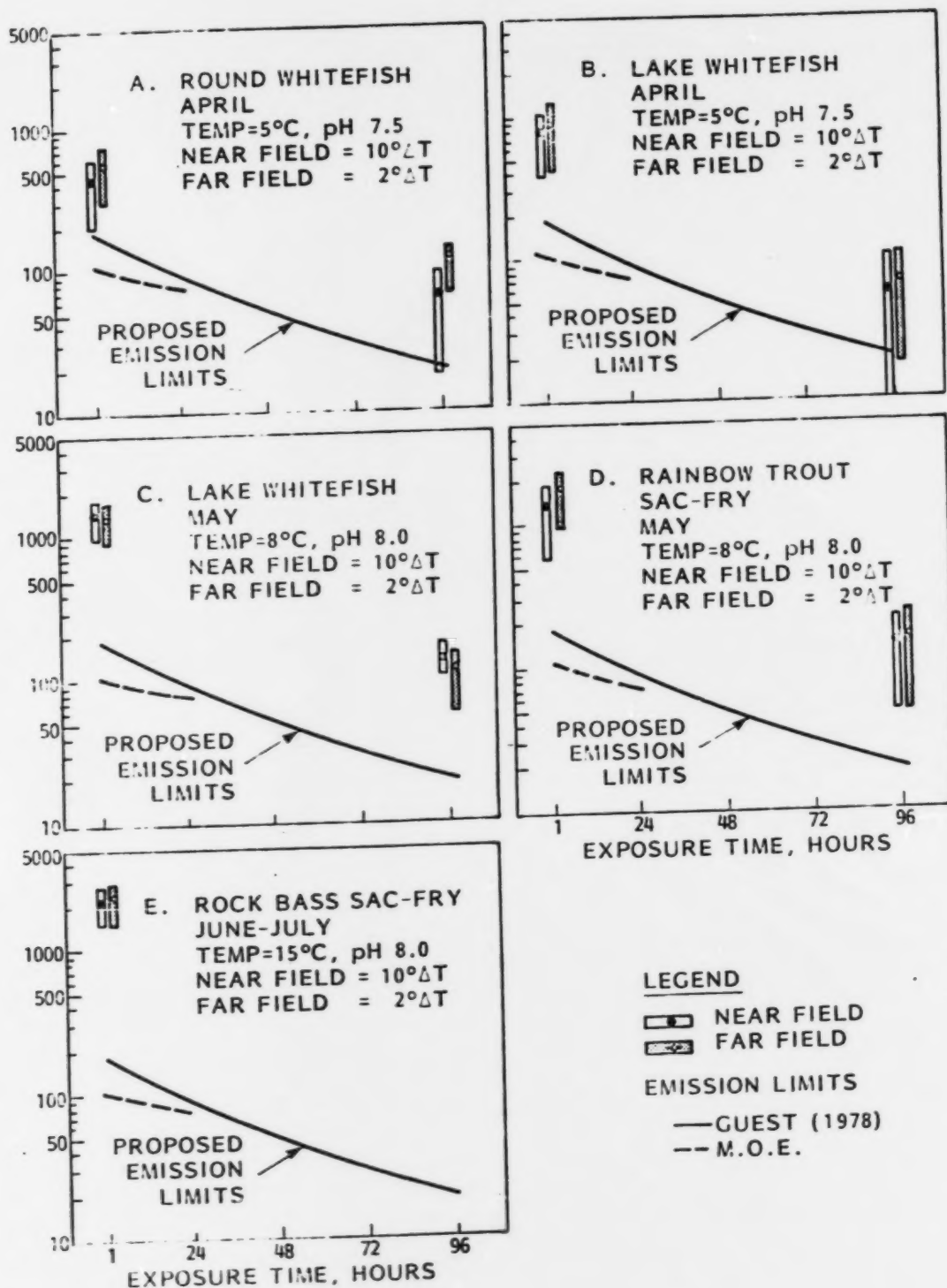


FIGURE 28
 COMPARISON OF SAC-FRY TOXICITY DATA
 WITH PROPOSED EMISSION LIMITS FOR
 BRUCE HEAVY WATER PLANT

round whitefish will hatch in the late April to mid-May period. In Figures 28A and 28B, results for the species are shown, assuming that exposure occurs in April. While the LC50 values are well above the proposed emission limits, the results suggest that some level of stress could be experienced by whitefish sac-fry exposed to H_2S for 96 hour periods. However, as the variability associated with these round whitefish and lake whitefish models approximates 10% and 13%, respectively, great emphasis should not be placed on the predicted LC10 values. Also, it should be pointed out that the pH (7.5) assumed for April is probably somewhat lower than the true pH at this time of year. As previously indicated, the 96 hour results for round whitefish sac-fry at higher pH levels were considered unreliable. Results are available for lake whitefish at pH 8.0. For the May exposure period (Figure 28C) these results indicate that despite higher ambient temperatures, the toxic levels of H_2S at the assumed pH of 8.0 are well above the proposed emission limits. Similar results were obtained for rainbow trout sac-fry (Figure 28D) assuming a May exposure period and a pH level of 8.0. Due to limitations of time and availability of sac-fry, rock bass were tested only in 1 hour tests. These results (Figure 28E) suggest that toxic H_2S levels for this species are approximately an order of magnitude above the proposed emission limits, despite assumed temperatures as high as 27°C.

In summary, the results obtained in this study indicate that the proposed maximum emission limits for the Bruce Heavy Water Plants are substantially below the LC50 values at the prevailing pH and temperature levels for various seasons of the year. Only in the case of round whitefish and lake whitefish sac-fry exposed during April was there an indication that some mortality could result from the maximum emission levels. However, the pH level assumed for this period (pH 7.5) may be unrealistically low. If the true pH is somewhat higher (near 8.0) then toxic levels of H_2S for whitefish sac-fry would be substantially above the proposed emission limits.

SUMMARY

1. Short-term laboratory bioassays (1 hour and 96 hour) were conducted to determine the toxicity of H_2S to sac-fry of various Great Lakes fish species (round whitefish, lake whitefish, rainbow trout, rock bass) under the seasonal pH and temperature conditions likely to be experienced in the BHWP discharge to Lake Huron. Similar tests were also conducted with rainbow trout juveniles. Experiments were arranged in partial factorial designs (minimum of 33 test points) to determine the effect of H_2S concentration on survival over a range of temperatures. Separate experiments were run at three pH levels (7.5, 8.0, 8.5) arranged to span the seasonal range expected in Lake Huron.
2. Response surface models were fitted to the results from each experiment. Separate models were fitted for total H_2S and aqueous H_2S levels. Contour plots from these models

defined the relationship between temperature and the H_2S concentration causing mortality of 50% of test fish (LC50) at each experimental pH level. The LC10 and LC90 contours were also calculated to determine the range of H_2S levels over which mortality occurred. Examination of the models suggested that the best correlation between observed and predicted values (residuals) occurred for the LC50 estimates in the region of the experimental design centre (mid-range temperature). The average residual for all experiments approximated 10%. The range of H_2S concentrations indicated by the computed LC10 to LC90 contours of the models appeared to provide a reasonable estimate of the H_2S range from minimal mortality to nearly complete mortality, as observed lethal boundaries were somewhat more abrupt than those suggested by the models.

3. Toxic levels of H_2S varied with exposure time, pH, temperature, fish species, life stage, and to some extent with the particular group of test fish examined (two-year classes of rainbow trout sac-fry). In general, results suggest the following order of sensitivity to H_2S over most pH and temperature conditions: round whitefish sac-fry $\geq$ lake whitefish sac-fry $>$ rainbow trout sac-fry $>$ rainbow trout juveniles $\geq$ rock bass sac-fry.
4. Experiences of the present study indicate that some difficulties exist in conducting bioassays, especially longer tests (96 hour) with sac-fry. The sac-fry stages are available for only a brief time period, necessitating careful scheduling if studies are to be completed using healthy, newly hatched fry. The limited yolk-sac reserves preclude extensive thermal acclimation prior to testing (a three-day period was used in the present study). In this study, it was necessary to repeat some tests with round whitefish sac-fry, resulting in subsequent tests being run with fish which had partially resorbed their yolk sacs. These fry appeared to have insufficient yolk reserves to survive the combined acclimation/test periods in 96 hour bioassays at warm temperatures. If such problems are anticipated, some relief may be obtained through incubation of eggs at various temperatures to extend the hatching period. However, excessively low or high temperatures should be avoided to maintain the quality of hatched fry. Present experiments with rock bass suggested that the sac-fry used in the pH 8.5 tests (last group tested) were incubated at excessively low temperatures as their subsequent survival was poor when these low temperatures were maintained after hatch.
5. As test temperatures increased, fish displayed slightly greater sensitivity to given levels of total H_2S . This occurred despite a decline in the proportion of the most toxic H_2S (aqueous) form due to greater dissociation at the warm temperatures. When results were considered in terms of aqueous H_2S levels, a more pronounced increase in

sensitivity was apparent at warm temperatures, although some variability still persisted. A regression was fitted relating the decline in the $H_2S_{(aq)}$ LC50 to temperature for all species/life stages tested in this study. Separate regressions for 1 hour and 96 hour bioassays yielded similar slopes, with Q_{10} values approximating -1.8. The increased toxicity of $H_2S_{(aq)}$ with temperature closely approximates the basal metabolic rate increase expected for thermally acclimated fish (exposed to non-lethal temperatures). Thus, toxicity increases with temperature may be largely a function of the rate at which H_2S passes through the fish gill. Higher metabolic demands will result in increased gill ventilation, increasing the amount of H_2S solution coming in contact with the gill at warm temperatures.

6. Over the pH range of 7.5 to 8.5, substantially greater concentrations of total H_2S were required to elicit a toxic response (a given level of mortality). This may be largely explained by the dissociation of H_2S over this pH range, as much more of the highly toxic aqueous form is present at the lower pH. However, when results were computed in terms of aqueous H_2S levels, an increase in sensitivity was apparent as pH increased from 7.5 to 8.5. Generalized regressions (fitted for all species/life stages) indicated a comparable increase in sensitivity for both 1 hour and 96 hour tests. The aqueous H_2S LC50 values at pH 8.5 were about 60% lower than those at pH 7.5. Similar results have been reported by Smith et al (1976) for the fathead minnow. These results suggest that the HS^- ion, which is abundant at high pH levels, may contribute to the toxicity of a H_2S solution. Calculations based on present results suggest that the HS^- ion is about one-twelfth as toxic as the dissolved H_2S molecule. This difference may be due to the polarity of the ion, which will not pass through membranes (ie, the fish gill) as easily as the H_2S molecule.
7. Comparison of present results with those obtained in 1 hour and 96 hour bioassays on the same (or closely related) species/life stages by MacLaren (1977) indicated that toxic H_2S levels of the present study were substantially higher. Direct comparisons were possible for 18 bioassay tests. In 15 tests, MacLaren's LC50 values occurred at lower H_2S concentrations than those of the present study. In nine of these cases, MacLaren's LC50 occurred at H_2S levels below the LC10 of the present study, and in most of these tests, MacLaren obtained nearly total mortality at or below the present LC10 levels. There is no obvious reason for the differences between the present results and those reported by MacLaren. However, it should be noted that the present response surface models are based on substantially more data than the MacLaren results. Also, as previously mentioned, the fragile nature of sac-fry can easily result in somewhat unhealthy fry being used in bioassay tests, producing low LC50 values.

8. Toxic levels of H_2S indicated by the present study were compared with maximum emission limits proposed by Guest (1978) for the Bruce Heavy Water Plants, considering the time of year that each species/life stage might be present in the BHWP discharge area, and the prevailing seasonal pH and temperature levels. These comparisons suggested that in most cases, the proposed maximum emission limits were substantially lower than the H_2S levels causing mortality in the present tests. Only in the case of lake whitefish or round whitefish sac-fry exposed in April (early hatch) was there some indication that mortality might occur in immediate discharge areas, as the maximum emission limits approximated the LC10 values of the 96 hour tests. However, the pH assumed at this time (pH 7.5) may be unrealistically low. If a higher pH level (8.0) were assumed, the H_2S concentration causing mortality in these species would be well above the maximum emission limit.

The majority of laboratory work in this study (rearing fish, conducting bioassays, H_2S analysis, computer analysis of data) was done by Mr. P.U. Niklas and Ms. G.A. Watson. The author also acknowledges the work of Mr. Niklas in the design and construction of the apparatus, development of test procedures, and preparation of portions of this report.

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APPENDIX 1 - ANALYSIS OF WATER SAMPLES FROM BIOASSAY SYSTEM

<u>Parameter</u>	<u>Jan 17/80</u>	<u>Feb 6/80</u>
pH	7.4	7.55
<u>Alkalinity</u>		
mg/L CaCO ₃ , HCO ₃	68.0	80.0
<u>Hardness</u>		
mg/L as CaCO ₃	135	133
Sp. Cond. ms/m	34.5	40.5
Turbidity (NTU)	0.42	0.78
Total PO ₄ , mg/L P	<0.01	0.011
Residual Chlorine mg/L	<0.02	<0.04
Cl mg/L	45.0	45.3
SO ₄ mg/L	29.3	28.0
NO ₃ mg/L	2.2	2.12
Ca mg/L	40.6	39.9
Mg mg/L	8.3	8.2
Fe mg/L	<0.04	0.05
Mn mg/L	<0.02	<0.02
Zn mg/L	<0.01	<0.01
Pb µg/L	<2.0	<2.0
Cd µg/L	<0.05	2.52
Cu µg/L	6.4	7.03
Cr µg/L	<0.5	<0.5
Hg µg/L	<0.2	<0.2
Ni		3.1
Na		21.1
K		0.92

APPENDIX 2A

RAINBOW TROUT SAC-FRY (GROUP 1)
 1 HOUR BIOASSAY DATA AT pH 7.5

Design H ₂ S Level	Design Temp °C	Observed Temp °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	% Survival (N=10)
0 (Control)	4.0	5.5	7.55	<23.27	<7.96	100 100 100
	14.0	14.0	7.57	0	0	100 100 100
	24.0	23.4	7.62	<23.27	<4.79	100 100 100
1	8.0	7.1 7.0 7.0	7.49 7.51 7.47	210.09 144.70 200.75	75.54 50.63 74.50	100 100 100
	20.0	20.3 20.3 20.2	7.53 7.50 7.49	154.05 172.73 163.39	39.42 46.51 44.82	100 100 100
2	4.0	4.7 4.8 4.8	7.52 7.57 7.52	465.41 396.91 471.64	169.93 134.14 171.76	100 100 100
	14.0	14.0 14.0 13.9	7.45 7.50 7.49	340.87 331.52 312.84	112.16 100.82 96.87	100 100 100
	24.0	24.1 24.1 24.2	7.55 7.59 7.59	378.23 384.46 424.93	87.28 82.59 91.12	100 100 100
3	8.0	7.0 7.0 7.1	7.47 7.48 7.46	549.48 589.96 415.59	203.93 215.79 156.11	100 100 100
	20.0	20.3 20.3 20.3	7.58 7.55 7.47	350.21 387.57 446.73	82.16 95.82 126.46	100 100 100
4	14.0	13.9 13.9 13.9	7.46 7.46 7.44	976.05 826.60 957.37	316.84 268.32 320.52	100 100 90

APPENDIX 2A CONTINUED
 RAINBOW TROUT SAC-FRY (GROUP 1)
 1 HOUR BIOASSAY DATA AT pH 7.5 (SECOND TEST)

Design H ₂ S Level	Design Temp °C	Observed Temp °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	% Survival (n=10)
0 (Control)	4.0	5.5	7.65	<23.27	<6.80	100
						100
						100
	13.0	13.4	7.56	<23.27	<6.50	100
						100
	22.0	21.9	7.67	<23.27	<4.50	100
1	8.0	7.6	7.47	284.82	104.24	100
		7.7	7.49	213.21	75.59	100
		7.6	7.50	266.14	93.18	100
	18.0	18.0	7.60	216.32	51.25	100
		18.1	7.56	210.09	53.25	100
		18.0	7.56	222.55	56.52	100
2	4.0	4.2	7.54	468.53	168.30	100
		4.5	7.57	521.46	177.66	100
		4.6	7.57	499.66	169.77	100
	13.0	13.3	7.47	415.59	134.44	100
		13.4	7.50	372.00	114.55	100
		13.2	7.45	378.23	126.45	100
	22.0	22.2	7.50	549.48	143.01	100
		22.3	7.54	406.25	98.51	100
		22.3	7.54	403.14	97.75	100
3	8.0	8.0	7.44	1050.78	398.00	100
		8.0	7.45	1165.99	435.34	100
		8.3	7.46	979.17	357.90	100
	18.0	18.1	7.55	677.14	174.61	100
		18.0	7.55	549.48	141.97	100
		18.2	7.55	835.94	215.13	50
3+	18.0	18.2	7.54	954.26	249.81	50
		18.3	7.54	1159.76	303.01	30
		18.3	7.52	1116.17	301.66	10
4	13.0	13.4	7.45	1256.28	418.32	30
		13.4	7.45	1200.24	399.66	70
		13.3	7.48	1256.28	400.09	60
4+	13.0	13.8	7.38	1807.40	663.16	0
		13.8	7.42	1804.29	623.90	0
		13.7	7.47	1773.15	568.96	10

APPENDIX 2A CONTINUED
RAINBOW TROUT SAC-FRY (GROUP 1.)
1 HOUR BIOASSAY DATA AT pH 8.0

Design H ₂ S Level	Design Temp °C	Observed Temp °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	% Survival (N=10)
0 (Control)	4.0	5.8	8.10	<43.48	<5.50	100
						100
						100
	13.0	12.8	8.16	<43.48	<3.92	100
						100
						100
1	22.0	21.2	8.19	<31.03	<2.13	100
						100
						100
	8.0	7.8	8.03	487.70	66.49	100
		7.7	8.06	371.46	47.85	100
		7.7	8.04	442.03	59.26	100
2	18.0	17.9	8.18	350.70	26.54	100
		17.9	8.15	404.67	32.64	100
		17.9	8.17	417.12	32.25	100
	4.0	5.0	7.94	956.83	169.97	100
		5.0	8.01	873.79	135.70	100
		5.1	7.99	998.34	160.63	100
3	13.0	12.8	8.04	728.49	84.19	100
		12.9	8.03	678.67	79.83	100
		12.6	8.06	620.55	69.22	100
	22.0	21.6	8.06	765.85	68.55	100
		21.5	8.06	836.43	75.03	100
		21.5	8.04	832.28	77.85	100
4	8.0	7.8	7.92	1201.77	203.08	90
		7.7	7.92	1388.59	235.36	80
		8.0	7.97	958.34	152.24	100
	18.0	17.9	8.02	1197.62	126.74	40
		17.9	7.98	1371.98	157.60	10
		18.0	8.03	1201.77	124.29	50
5	13.0	12.7	7.88	1886.72	300.44	40
		12.8	7.84	1917.91	329.06	20
		12.7	7.79	2260.41	427.28	30

APPENDIX 2A CONTINUED
RAINBOW TROUT SAC-FRY (GROUP 1)
1 HOUR BIOASSAY AT pH 8.5

Design H ₂ S Level	Design Temp °C	Observed Temp °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	% Survival (n=10)
0 (Control)	4.0	6.5	8.51	<54.35	<2.83	100 100 100
		12.7	8.66	<38.79	<1.18	100 100 100
		21.7	8.66	<54.35	<1.31	100 100 100
	13.0	12.7	8.66	<38.79	<1.18	100 100 100
		21.7	8.66	<54.35	<1.31	100 100 100
		21.7	8.66	<54.35	<1.31	100 100 100
1	8.0	8.0	8.59	521.40	21.57	100
		7.7	8.61	412.43	16.50	100
		7.7	8.60	536.97	21.96	100
	18.0	18.0	8.68	474.70	11.95	100
		18.0	8.64	448.75	12.36	100
		18.0	8.66	474.70	12.50	100
2	4.0	4.9	8.50	863.91	48.70	100
		5.0	8.47	801.63	48.04	100
		5.2	8.46	1004.02	61.03	100
	13.0	12.7	8.58	786.07	28.63	100
		12.8	8.60	723.79	25.14	100
		12.5	8.57	749.74	28.09	100
	22.0	22.0	8.59	754.93	21.09	100
		21.9	8.58	972.89	27.85	100
		21.9	8.59	941.75	26.37	100
3	8.0	8.0	8.43	1548.91	90.96	100
		8.0	8.42	1564.48	93.89	100
		8.5	8.46	1471.07	79.59	100
	18.0	8.0	8.20	2720.64	260.65	90
		8.0	8.21	2902.27	272.31	60
		8.7	8.18	2815.08	274.92	60
	18.0	18.0	8.53	1611.19	56.70	90
		18.0	8.51	1471.07	54.12	100
		18.0	8.42	1678.65	75.34	100
3+	18.0	18.0	8.22	2887.74	200.17	10
		18.1	8.20	3203.77	231.22	10
		18.0	8.27	2858.68	177.95	10
4	13.0	12.7	8.33	2607.56	164.24	50
		12.7	8.31	2374.04	156.12	60
		12.6	8.21	2695.78	220.06	40
4+	13.0	12.8	8.05	4333.52	490.72	10
		12.7	8.05	3984.79	452.47	0
		12.6	8.02	4384.37	530.57	0

APPENDIX 2B - ANALYSIS OF RAINBOW TROUT SAC-FRY (GROUP 1)
BIOASSAY DATA. TOTAL H₂S AT PH 7.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	39951.1543	5	7990.23	77.06 *
LINEAR	27034.6143	2	13517.31	130.37 *
X1 LINEAR	2426.7780	1	2426.78	23.41 *
X2 LINEAR	2551.3003	1	2551.30	246.43 *
QUADRATIC	6841.2400	2	3420.62	32.99 *
X1 QUAD.	9.8737	1	9.87	.10
X2 QUAD.	6787.2192	1	5787.22	65.46 *
X1 * X2	3647.3953	1	3647.40	35.18 *
RESIDUAL	6843.2876	66	103.69	
TOTAL	46794.4419	71		

REGRESSION:

$$Y = 85.8327 + 1.23402X_1 + 0.0717383X_2 - 0.0121104X_1^2 - 0.00003916X_2^2 - 0.00420756X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean Residual for Model (72 Data Points) = 6.86%

* Indicates significance at 0.05 probability level

APPENDIX 2C - ANALYSIS OF RAINBOW TROUT SAC-FRY (GROUP 1)
BIOASSAY DATA. TOTAL H₂S at PH 8.0 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	18718.2537	5	3743.65	20.15 *
LINEAR	13199.3712	2	6599.69	35.53 *
X ₁ LINEAR	1515.9311	1	1515.93	8.16 *
X ₂ LINEAR	12385.3195	1	12385.32	66.68 *
QUADRATIC	2183.8574	2	1091.93	5.88 *
X ₁ QUAD.	103.5303	1	103.54	.56
X ₂ QUAD.	1800.2601	1	1800.26	9.69 *
X ₁ * X ₂	1497.0696	1	1497.07	8.06 *
RESIDUAL	5015.0789	27	185.74	
TOTAL	23733.3325	32		

REGRESSION:

$$Y = 104.332 - 1.27762X_1 + 0.0339436X_2 + 0.071224X_1^2 - 0.00001807X_2^2 - 0.00264624X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean Residual for Model (33 Data Points) = 8.14%

* Indicates significance at 0.05 probability level

APPENDIX 2D - ANALYSIS OF RAINBOW TROUT SAC-FRY (GROUP 1)
BIOASSAY DATA. TOTAL H₂S AT PH 8.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	43513.0342	5	8702.61	71.77 *
LINEAR	31599.8662	2	15799.93	130.29 *
X1 LINEAR	2082.5519	1	2082.55	17.17 *
X2 LINEAR	30280.2483	1	30280.25	249.70 *
QUADRATIC	3120.7812	2	1560.39	12.87 *
X1 QUAD.	3.7435	1	3.74	.03
X2 QUAD.	3058.7416	1	3058.74	25.22 *
X1 X2	2660.2365	1	2660.24	21.94 *
RESIDUAL	4365.5352	36	121.26	
TOTAL	47878.5693	41		

REGRESSION:

$$Y = 93.7106 + 0.510005X_1 + 0.0202052X_2 + 0.0132808X_1^2 - 0.00000562911X_2^2 - 0.00170313X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean Residual for Model (42 Data Points) = 7.56%

* Indicates significance at 0.05 probability level

APPENDIX 2E - ANALYSIS OF RAINBOW TROUT SAC-FRY (GROUP 1)
BOIASSAY DATA. AQUEOUS H₂S AT pH 7.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	37687.5967	5	7537.52	54.63 *
LINEAR	27380.5876	2	13690.29	99.22 *
X1 LINEAR	5133.3006	1	5133.30	37.20 *
X2 LINEAR	26461.0103	1	26461.01	191.77 *
QUADRATIC	2875.9513	2	1437.98	10.42 *
X1 QUAD.	86.5897	1	86.59	.63
X2 QUAD.	2820.2624	1	2820.26	20.44 *
X1 * X2	6468.2654	1	6468.27	46.88 *
RESIDUAL	9106.8452	65	137.98	
TOTAL	46794.4419	71		

REGRESSION:

$$Y = 82.5368 + 2.04694X_1 + 0.215345X_2 - 0.0368603X_1^2 - 0.000208857X_2^2 - 0.0179599X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean Residual for Model (72 Data Points) = 8.17%

* Indicates significance at 0.05 probability level

APPENDIX 2F - ANALYSIS OF RAINBOW TROUT SAC-FRY (GROUP 1)
BIOASSAY DATA. AQUEOUS H₂S AT PH 8.0 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	18461.8918	5	3692.38	18.91 *
LINEAR	14570.8005	2	7285.40	37.32 *
X1 LINEAR	4237.8267	1	4237.83	21.71 *
X2 LINEAR	13681.7114	1	13681.71	70.08 *
QUADRATIC	149.3838	2	74.69	.38
X1 QUAD.	93.2879	1	93.29	.48
X2 QUAD.	77.5872	1	77.59	.40
X1 * X2	3384.0695	1	3384.07	17.33 *
RESIDUAL	5271.4407	27	195.24	
TOTAL	23733.3325	32		

REGRESSION:

$$Y = 107.790 - 1.21274X_1 + 0.134313X_2 + 0.0728284X_1^2 + 0.000116468X_2^2 - 0.0303951X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean Residual for Model (33 Data Points) = 9.44%

* Indicates significance at 0.05 probability level

APPENDIX 2G - ANALYSIS OF RAINBOW TROUT SAC-FRY (GROUP 1)
BIOASSAY DATA. AQUEOUS H₂S AT pH 8.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	44775.8921	5	8955.16	103.91 *
LINEAR	35108.0334	2	17554.02	203.48 *
X1 LINEAR	6474.1198	1	6474.12	75.12 *
X2 LINEAR	13940.5801	1	13940.58	1593.91 *
QUADRATIC	901.7163	2	450.86	5.23 *
X1 QUAD.	.4703	1	.47	.01
X2 QUAD.	901.1412	1	901.14	10.46 *
X1 . X2	6087.8377	1	6087.84	70.44 *
RESIDUAL	3102.6772	16	193.92	
TOTAL	47878.5693	41		

REGRESSION:

$$Y = 100.101 + 0.556359X_1 + 0.0888637X_2 - 0.00472307X_1^2 + 0.000222771X_2^2 - 0.0324435X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean Residual for Model (42 Data Points) = 6.53%

* Indicates significance at 0.05 probability level

APPENDIX 3A
RAINBOW TROUT SAC-FRY (GROUP 2)
1 HOUR BIOASSAY AT pH 7.5

Design H ₂ S Level	Design Temp °C	Observed Temp °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	% Survival
0 (Control)	4.0	4.9	7.63	0	0	100
						100
						100
	12.0	11.5	7.63	0	0	100
						100
						100
1	20.0	19.9	7.71	0	0	100
						100
						100
	8.0	7.9	7.45	538.08	201.35	100
		8.0	7.50	538.08	186.63	100
		7.9	7.58	538.08	165.26	100
2	16.0	16.1	7.45	418.06	132.06	100
		16.0	7.41	435.21	146.55	100
		16.0	7.42	366.62	121.58	100
	4.0	4.4	7.51	1069.59	399.31	100
		4.3	7.52	795.26	293.38	100
		4.4	7.53	1069.59	387.85	100
3	12.0	11.7	7.46	983.86	334.06	100
		12.2	7.47	1052.44	348.27	90
		11.7	7.49	966.72	313.44	80
	20.0	19.9	7.61	880.99	197.48	50
		20.0	7.66	760.97	155.51	90
		20.0	7.66	1001.01	204.56	70
4	8.0	8.1	7.42	1361.06	529.20	90
		7.9	7.47	1189.60	432.40	100
		8.1	7.46	1532.52	562.68	90
	16.0	16.0	7.45	1069.59	338.51	70
		16.0	7.50	932.42	272.38	80
		16.0	7.44	1206.75	387.96	80
4	12.0	12.0	7.44	1944.01	676.12	20
		12.0	7.45	1801.13	617.05	18
		12.0	7.47	2012.59	668.78	30

APPENDIX 3A CONTINUED
RAINBOW TROUT SAC-FRY (GROUP 2)
1 HOUR BIOASSAY AT pH 8.0

Design H ₂ S Level	Design Temp °C	Observed Temp °C	Observed pH	Total Sulphide	Aqueous H ₂ S	% Survival
0 (Control)	4.0	5.0	8.20	0	0	100
						100
						100
	12.0	11.3	8.21	0	0	100
						100
						100
	20.0	20.1	8.22	0	0	100
						100
						100
1	8.0	8.2	8.03	812.41	109.39	100
		8.0	8.04	692.39	91.96	100
		8.3	8.4	749.54	98.63	100
		16.0	8.00	606.66	69.69	100
	16.0	16.2	8.02	555.22	61.22	100
		16.1	8.06	520.93	53.03	100
2	4.0	4.5	7.97	1583.95	270.25	100
		4.4	8.04	1223.90	183.00	100
		4.5	7.97	1601.10	273.17	100
		12.0	7.96	1378.21	192.73	100
	12.0	11.7	7.96	1372.49	190.89	90
		11.9	7.91	1669.68	256.89	80
		11.8	8.04	1241.04	120.03	100
		20.0	8.09	1086.73	94.67	100
	20.0	20.0	8.07	1378.21	125.21	80
3	8.0	8.3	7.91	2029.74	344.47	60
		8.3	7.92	1984.01	330.32	70
		8.2	7.91	2166.90	368.83	50
		16.0	7.90	1738.26	244.11	90
	16.0	16.2	7.93	1721.12	227.71	90
		16.2	7.89	2029.74	290.74	50
4	12.0	11.6	7.79	2578.39	501.10	30
		11.9	7.78	2646.97	520.06	20
		11.8	7.76	2755.56	563.12	10

APPENDIX 3A CONTINUED
RAINBOW TROUT SAC-FRY (GROUP 2)
1 HOUR BIOASSAY AT pH 8.5

Design H ₂ S Level	Design Temp °C	Observed Temp °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	% Survival
0 (Control)	4.0	5.0	8.76	0	0	100
						100
						100
	12.0	11.0	8.78	0	0	100
						100
						100
1	20.0	19.8	8.75	0	0	100
						100
						100
	8.0	7.7	8.66	1039.68	37.23	100
		7.8	8.68	911.09	31.10	100
		7.7	8.69	925.37	31.00	100
2	16.0	15.6	8.73	858.70	20.60	100
		15.7	8.76	725.34	16.22	100
		15.7	8.78	739.63	15.81	100
	4.0	4.5	8.45	2182.71	139.20	100
		4.5	8.52	1839.80	100.82	100
		4.5	8.49	2316.06	135.47	100
3	12.0	11.5	8.56	2039.83	80.52	100
		11.6	8.58	2011.25	75.72	100
		11.5	8.58	2039.83	77.03	100
	20.0	19.7	8.55	2025.54	65.35	100
		19.7	8.60	1573.09	45.39	100
		19.7	8.54	2239.86	73.89	100
3+	8.0	7.8	8.45	3154.29	178.58	100
		7.8	8.45	2897.10	164.02	100
		7.9	8.39	3611.50	232.02	100
	16.0	15.7	8.52	2839.95	108.55	100
		15.7	8.55	2639.92	94.41	100
		15.6	8.45	3640.07	162.82	90
4	8.0	8.2	8.43	3725.80	217.34	60
		8.1	8.33	3640.07	264.21	60
		8.2	8.10	4535.45	530.42	50
	16.0	15.8	8.37	3782.95	200.55	50
		15.8	8.46	3192.39	138.94	60
		15.8	8.34	4040.14	228.63	60
4	12.0	11.5	8.32	3992.51	266.13	50
		11.6	8.29	4325.89	306.60	10
		11.5	8.31	4240.17	288.77	30
4+	12.0	11.5	8.04	5634.42	675.99	30
		11.8	7.92	6877.90	1037.77	0
		11.4	8.00	5677.72	739.24	20

APPENDIX 3B - ANALYSIS OF RAINBOW TROUT SAC-FRY (GROUP 2)
BIOASSAY DATA. TOTAL H₂S AT PH 7.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	15753.0110	5	3150.60	37.79 *
LINEAR	11492.4731	2	5746.24	68.93 *
X1 LINEAR	2648.6735	1	2648.67	31.77 *
X2 LINEAR	9961.7548	1	9961.75	119.50 *
QUADRATIC	3440.4096	2	1720.20	20.63 *
X1 QUAD.	75.1795	1	75.18	.90
X2 QUAD.	3438.9872	1	3438.99	41.28 *
X1 * X2	1676.6941	1	1676.69	20.11 *
RESIDUAL	2250.8672	27	83.37	
TOTAL	18003.8782	32		

REGRESSION:

$$Y = 88.1972 + 1.93036X_1 + 0.0489654X_2 - 0.0665765X_1^2 - 0.0000270869X_2^2 - 0.00289796X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean Residual for Model (33 Data Points) = 6.11%

* Indicates significance at 0.05 probability level

APPENDIX 3C - ANALYSIS OF RAINBOW TROUT SAC-FRY (GROUP 2)
BIOASSAY DATA. TOTAL H₂S AT PH 8.0 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	19890.9707	5	3978.19	95.29
LINEAR	10972.5513	2	5486.28	131.41
X1 LINEAR	117.7149	1	117.71	2.82
X2 LINEAR	10959.1492	1	10959.15	262.50
QUADRATIC	6615.6312	2	3307.82	79.23
X1 QUAD.	68.1597	1	68.16	1.63
X2 QUAD.	6043.2937	1	6043.29	144.78
X1 * X2	43.5780	1	43.58	1.04
RESIDUAL	1127.2104	27	41.75	
TOTAL	21010.1612	32		

REGRESSION:

$$Y = 106.973 - 1.65371X_1 + 0.0274912X_2 + 0.0642825X_1^2 - 0.0000198175X_2^2 - 0.000311548X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean Residual for Model (33 Data Points) = 4.37%

* Indicates significance at 0.05 probability level

APPENDIX 3D - ANALYSIS OF RAINBOW TROUT SAC-FRY (GROUP 2)
BIOASSAY DATA. TOTAL H₂S AT PH 8.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	28621.5174	5	5724.30	29.48*
LINEAR	17005.7939	2	8502.90	43.79*
X1 LINEAR	48.7929	1	48.79	.25
X2 LINEAR	16970.6406	1	16970.64	87.40*
QUADRATIC	4815.7072	2	2407.85	12.40*
X1 QUAD.	199.0932	1	199.09	1.03
X2 QUAD.	4441.9725	1	4441.97	22.88*
X1 * X2	23.7009	1	23.70	.12
RESIDUAL	6990.3857	34	205.61	
TOTAL	35611.9033	41		

REGRESSION:

$$Y = 115.111 - 2.73104X_1 + 0.00381482X_2 + 0.11175X_1^2 - 0.00000282641X_2^2 - 0.000121528X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean Residual for Model (42 Data Points) = 8.76%

* Indicates significance at 0.05 probability level

APPENDIX 3E - ANALYSIS OF RAINBOW TROUT SAC-FRY (GROUP 2)
BIOASSAY DATA. AQUEOUS H₂S AT PH 7.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	15321.6304	5	3064.33	30.85 *
LINEAR	11837.4026	2	5918.70	59.58 *
X1 LINEAR	4804.8630	1	4804.86	48.37 *
X2 LINEAR	10634.2855	1	10634.29	107.05 *
QUADRATIC	2632.8184	2	1316.41	13.25 *
X1 QUAD.	437.3898	1	437.39	4.40 *
X2 QUAD.	2366.3226	1	2366.32	23.82 *
X1 * X2	2837.4928	1	2837.49	28.56 *
RESIDUAL	2682.2478	27		
TOTAL	18003.8782	32		

REGRESSION:

$$Y = 75.753 + 4.5492X_1 + 0.163577X_2 - 0.171906X_1^2 - 0.000195408X_2^2 - 0.0125098X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean Residual for Model (33 Data Points) = 7.04%

* Indicates significance at 0.05 probability level

APPENDIX 3F - ANALYSIS OF RAINBOW TROUT SAC-FRY (GROUP 2)
BIOASSAY DATA. AQUEOUS H₂S AT PH 8.0 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	19370.9553	5	3874.19	63.50 *
LINEAR	12656.4919	2	6428.25	105.37 *
X1 LINEAR	440.2503	1	440.26	7.22 *
X2 LINEAR	12511.0312	1	12511.03	205.07 *
QUADRATIC	2452.2070	2	1226.10	20.10 *
X1 QUAD.	16.9172	1	16.92	.28
X2 QUAD.	2404.0027	1	2404.00	39.40 *
X1 * X2	205.2823	1	205.28	3.36
RESIDUAL	1647.2258	27	61.01	
TOTAL	21018.1812	32		

REGRESSION:

$$Y = 105.623 - 0.875219X_1 + 0.0633114X_2 + 0.0337645X_1^2 - 0.000310476X_2^2 - 0.00485260X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean Residual for Model (33 Data Points) = 4.758

* Indicates significance at 0.05 probability level

APPENDIX 3G - ANALYSIS OF RAINBOW TROUT SAC-FRY (GROUP 2)
BIOASSAY DATA. AQUEOUS H₂S AT PH 8.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSC	F
REGRESSION	20581.7503	4	5145.435	29.27 *
LINEAR	20241.6240	3	6747.213	51.83 *
X1 LINEAR	1288.1687	1	1288.17	6.60 *
X2 LINEAR	20053.3303	1	20053.33	102.60 *
QUADRATIC	2024.3988	1	2024.40	5.14 *
X3 QUAD.	116.0024	1	116.00	.50
X2 QUAD.	1571.7967	1	1571.80	8.05 *
X1 * X2	939.9820	1	939.98	4.81 *
RESIDUAL	7030.1440	36	195.29	
TOTAL	27611.9013	41		

REGRESSION:

$$Y = 114.338 - 1.90462X_1 - 0.0461839X_2 + 0.086807X_1^2 + 0.000095021X_2^2 - 0.0126996X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean Residual for Model (42 Data Points) = 9.79%

* Indicates significance at 0.05 probability level

APPENDIX 4A
RAINBOW TROUT FRY
1 HOUR BIOASSAY AT pH 7.5

Design H ₂ S Level	Design Temp °C	Observed Temp °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	% Survival
0 (Control)	4.0	5.0	7.67	<5	<1.43	100
						100
						100
	14.0	14.0	7.74	<5	<1.00	100
						100
						100
1	24.0	23.5	7.86	<5	<0.65	100
						100
						100
	8.0	8.0	7.57	187.54	58.38	100
		8.1	7.54	165.32	53.81	100
		7.8	7.55	162.85	52.57	100
2	20.0	20.0	7.72	150.50	27.51	100
		19.9	7.73	143.09	25.72	100
		19.9	7.74	143.09	25.24	100
	4.0	4.7	7.59	409.78	134.66	90
		4.5	7.58	422.12	141.64	100
		4.7	7.59	387.55	137.39	100
3	14.0	14.2	7.56	412.24	113.17	100
		14.4	7.60	434.47	110.99	100
		14.1	7.58	385.08	102.44	100
	24.0	24.2	7.76	343.11	53.46	90
		24.3	7.77	301.13	45.92	100
		24.3	7.78	335.70	50.21	100
4	8.0	8.1	7.54	602.38	196.07	60
		8.0	7.52	691.28	232.61	40
		8.3	7.52	730.79	244.16	30
	20.0	20.0	7.68	594.97	117.19	30
		20.0	7.69	634.48	122.68	10
		20.0	7.69	639.42	123.64	0
4	14.0	14.2	7.59	846.84	221.02	0
		14.1	7.67	777.70	176.98	0
		14.0	7.68	886.35	198.59	0

APPENDIX 4A CONTINUED
RAINBOW TROUT FRY
1 HOUR BIOASSAY AT pH 8.0

Design H ₂ S Level	Design Temp °C	Observed Temp °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	% Survival
0 (Control)	4.0	5.0	7.97	<5	<0.84	100 100 100
	14.0	14.0	8.00	<5	<0.61	100 100
	24.0	24.2	8.12	<5	<0.37	100 100 100
1	8.0	8.0	7.84	240.88	47.05	100
		8.0	7.91	201.87	34.56	100
		7.9	7.92	222.67	37.52	100
	20.0	19.8	8.03	220.07	21.83	100
		19.8	8.02	225.27	22.81	100
		19.7	8.03	214.87	21.36	100
2	4.0	4.9	7.91	547.72	103.28	100
		4.9	7.84	576.32	123.61	100
		4.9	7.91	529.51	99.85	100
	14.0	14.0	7.85	537.31	87.76	100
		14.1	7.87	571.12	89.52	100
		13.8	7.89	568.52	86.36	100
	24.0	24.3	8.00	472.31	45.25	90
		24.3	8.00	435.90	41.76	100
		24.3	8.00	456.70	43.76	100
3	8.0	8.1	7.86	773.74	145.22	100
		8.0	7.84	844.15	164.88	100
		8.1	7.87	924.76	170.30	100
	20.0	19.7	7.93	799.95	97.61	60
		19.7	7.93	820.72	100.15	30
		19.8	7.94	838.95	100.10	40
4	14.0	13.8	7.83	1132.79	193.21	20
		13.9	7.84	1652.53	275.83	0
		13.8	7.90	1171.79	174.54	0

APPENDIX 4A CONTINUED
RAINBOW TROUT FRY
1 HOUR BIOASSAY AT pH 8.5

Design H ₂ S Level	Design Temp °C	Observed Temp °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	% Survival
0 (Control)	4.0	5.3	8.55	<5	<0.25	100
						100
						100
	14.0	13.5	8.63	<5	<0.16	100
						100
						100
	24.0	22.8	8.52	<5	<0.16	100
						100
						100
1	8.0	8.0	8.67	369.38	12.80	100
						100
						100
	20.0	7.8	8.69	334.31	11.16	100
		7.7	8.69	311.77	10.44	100
		19.5	8.65	361.87	9.38	100
		20.3	8.61	334.31	9.30	100
		20.3	8.61	316.78	8.81	100
2	4.0	4.8	8.95	715.04	45.08	100
						100
						100
	14.0	5.1	8.40	820.24	56.94	100
		4.9	8.43	722.56	47.38	100
		14.1	8.47	730.07	32.57	100
		14.1	8.47	785.18	35.03	100
		13.5	8.53	770.15	30.62	100
		24.0	8.43	737.58	28.12	100
	24.0	24.2	8.42	689.99	26.78	100
		24.4	8.41	702.52	27.75	100
3	8.0	8.2	8.54	1025.63	47.22	100
						80
						90
	20.0	8.0	8.51	1223.51	60.36	100
		7.5	8.53	1273.61	61.18	100
		19.5	8.51	1045.67	37.06	100
		19.5	8.49	1233.54	45.70	100
		20.3	8.45	1288.64	51.16	100
4	14.0	13.8	8.30	1778.30	115.84	20
		13.8	8.34	1591.27	95.08	10
		13.7	8.33	1604.63	98.25	10

APPENDIX 4B - ANALYSIS OF RAINBOW TROUT FRY BIOASSAY DATA.
TOTAL H₂S AT PH 7.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - TRANSFORMED DATA

SOURCE	SS	D.F.	MSS	APPROX. F	MLC
REGRESSION	28975.2332	1	5795.05	61.44	.528267-18 *
LINEAR	20001.5349	2	10000.77	106.02	.135570-15 *
X1 LINEAR	1196.0167	1	1196.02	12.68	.142436-02 *
X2 LINEAR	19547.6460	1	19547.65	207.23	.189888-15 *
QUADRATIC	5990.3542	2	2995.18	31.75	.138501-08 *
X1 QUAD.	.0524	1	.05	.00	.999647-00
X2 QUAD.	5521.1218	1	5521.12	58.53	.355802-08 *
X1 * X2	1394.6649	1	1394.66	14.79	.593701-03 *
TRANSFORM	1563.7214	1	1563.72	.00	.292100-03 *
RESIDUAL	2452.4890	26	94.33		
TOTAL	31427.7222	32			

RELATIVE LIKELIHOOD OF NO TRANSFORMATION VS. ML ESTIMATES = .20211-03

REGRESSION:

$$Y^{.6047} = 14.8834 + 0.0826378X_1 + 0.0218166X_2 + 0.000218675X_1^2 - 0.0000360783X_2^2 - 0.000796892X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean Residual for Model (33 Data Points) = 8.38%

* Indicates significance at 0.05 probability level

APPENDIX 4C - ANALYSIS OF RAINBOW TROUT FRY BIOASSAY DATA.
TOTAL H₂S AT PH 8.0 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - TRANSFORMED DATA

SOURCE	SS	D.F.	MSS	APPROX. F	MLF
REGRESSION	25116.1611	5	5023.63	23.97	.439252-12 X
LINEAR	16098.8928	2	8049.45	38.41	.140678-09 X
X1 LINEAR	2019.4295	1	2019.43	9.54	.550819-02 X
X2 LINEAR	15031.8810	1	15031.88	71.72	.325200-09 X
QUADRATIC	4368.6589	2	2184.33	10.42	.604230-04 X
X1 QUAD.	98.8778	1	98.88	.47	.743255+00
X2 QUAD.	4022.4713	1	4022.47	19.19	.109255-03 X
X1 * X2	2059.3195	1	2059.32	9.83	.504472-02 X
TRANSFORM	192.4929	1	192.49	.00	.563941+00
RESIDUAL	5449.1814	26	209.58		
TOTAL	30567.3425	32			

RELATIVE LIKELIHOOD OF NO TRANSFORMATION VS. ML ESTIMATES = .56394+00

REGRESSION:

$$Y = .8187 = 44.1071 - 0.36587X_1 + 0.0265997X_2 + 0.0206417X_1^2 - 0.0000214174X_2^2 - 0.00165513X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean Residual for Model (33 Data Points) = 9.66%

* Indicates significance at 0.05 probability level

APPENDIX 4D - ANALYSIS OF RAINBOW TROUT FRY BIOASSAY DATA.
TOTAL H₂S AT PH 8.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	16570.6313	5	3314.13	22.48 *
LINEAR	7615.3834	2	3807.69	25.83 *
X1 LINEAR	39.0674	1	39.07	.26
X2 LINEAR	7551.9077	1	7551.91	51.22 *
QUADRATIC	7722.1394	2	3861.07	26.19 *
X1 QUAD.	25.6232	1	25.62	.17
X2 QUAD.	6725.5953	1	6725.60	45.62 *
X1 * X2	84.4788	1	84.48	.57
RESIDUAL	3980.4833	27	147.44	
TOTAL	20551.5144	32		

REGRESSION:

$$Y = 103.284 - 0.997245X_1 + 0.0425874X_2 + 0.0280826X_1^2 - 0.0000542831X_2^2 + 0.000578495X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean Residual for Model (33 Data Points) = 8.05%

* Indicates significance at 0.05 probability level

APPENDIX 4E - ANALYSIS OF RAINBOW TROUT FRY BIOASSAY DATA.
AQUEOUS H₂S AT PH 7.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - TRANSFORMED DATA

SOURCE	SS	D.F.	MSS	APPROX. F	MLR
REGRESSION	29360.9312	5	5876.19	43.24	.311687-16 *
LINEAR	18295.9252	2	9147.91	67.32	.292974-13 *
X1 LINEAR	4439.0039	1	4439.00	62.10	.701142-09 *
X2 LINEAR	16602.6235	1	16602.62	122.18	.112980-12 *
QUADRATIC	133.9752	2	66.99	.49	.514655+00
X1 QUAD.	39.2803	1	39.28	.29	.R20747+00
X2 QUAD.	83.7174	1	83.72	.62	.658230+00
X1 * X2	6813.5074	1	6813.51	50.14	.827265-08 *
TRANSFORM	3952.8269	3	1317.61	.00	.204644-05 *
RESIDUAL	3261.3699	24	135.89		
TOTAL	32642.3010	32			

RELATIVE LIKELIHOOD OF NO TRANSFORMATION VS. ML ESTIMATES = .20464-05

REGRESSION:

$$Y^{0.6654} = 24.3585 - 0.000160658 X_1^{3.0} - 0.0000183806 X_2^{2.111} + 0.0000000193762 X_1^{6.0} - 0.000000000546803 X_2^{4.222} - 0.000000088244 X_1^{3.0} X_2^{2.111}$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean residual for Model (33 Data Points) = 8.39%

* Indicates significance at 0.05 probability level.

APPENDIX 4F - ANALYSIS OF RAINBOW TROUT FRY BIOASSAY DATA.
AQUEOUS H₂S AT PH 8.0 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	23817.7307	5	4763.55	20.19 *
LINEAR	16997.7388	2	8498.87	36.02 *
X1 LINEAR	6294.0831	1	6294.08	26.68 *
X2 LINEAR	16065.5791	1	16065.58	68.09 *
QUADRATIC	756.4969	2	378.25	1.60
X1 QUAD.	1.0373	1	1.04	.00
X2 QUAD.	756.2481	1	756.25	3.21
X1 X2	5748.0598	1	5748.06	24.36 *
RESIDUAL	6370.1465	27	235.93	
TOTAL	30187.8772	32		

REGRESSION:

$$Y = 92.8918 + 0.904633X_1 + 0.426218X_2 - 0.0053551X_1^2 - 0.000830944X_2^2 - 0.0452291X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean residual for Model (33 Data Points) = 10.65%

* Indicates significance at 0.05 probability level

APPENDIX 4G - ANALYSIS OF RAINBOW TROUT FRY BIOASSAY DATA.
 AQUEOUS H₂S AT PH 8.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	18460.8159	5	3692.16	47.68 *
LINEAR	10757.2643	2	5378.63	69.46 *
X1 LINEAR	62.1588	1	62.16	.80
X2 LINEAR	10551.6145	1	10551.61	136.27 *
QUADRATIC	5504.2820	2	2752.14	35.54 *
X1 QUAD.	59.8136	1	59.81	.77
X2 QUAD.	4900.2024	1	4900.20	63.28 *
X1 * X2	33.6847	1	33.68	.44
RESIDUAL	2090.6987	27	77.43	
TOTAL	20551.5146	32		

REGRESSION:

$$Y = 105.899 - 1.20671X_1 + 0.530565X_2 + 0.0425617X_1^2 - 0.0113313X_2^2 - 0.00769772X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean residual for Model (33 Data Points) = 4.58%

* Indicates significance at 0.05 probability level

APPENDIX 5A
RAINBOW TROUT JUVENILES
1 HOUR BIOASSAY AT pH 7.5

Design H ₂ S Level	Design Temp °C	Observed Temp °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	% Survival
0 (Control)	4.0	7.7	7.26	<5	<2.41	100 100 100
	12.0	12.5	7.17	<5	<2.47	100 100 100
	20.0	20.0	7.16	<5	<2.24	100 100 100
1	8.0	9.5 9.0 9.1	7.27 7.27 7.29	180.85 140.14 167.28	83.40 65.22 75.80	100 100 100
	16.0	16.3 16.3 16.2	7.28 7.25 7.35	113.01 113.01 158.24	45.71 47.60 58.07	100 100 100
2	4.0	7.0 6.7 6.7	7.34 7.37 7.40	393.43 384.39 429.62	174.38 164.90 177.08	100 100 100
	12.0	12.6 12.7 12.5	7.25 7.29 7.22	370.82 330.11 370.82	166.02 140.07 172.66	100 100 100
	20.0	20.0 20.0 20.1	7.26 7.32 7.35	330.11 343.68 316.54	129.46 123.64 108.71	100 100 100
3	8.0	8.5 8.9 9.0	7.34 7.38 7.35	732.66 800.48 746.23	314.97 323.48 313.42	100 100 100
	16.0	16.2 16.3 16.3	7.29 7.34 7.29	927.15 936.19 931.67	370.50 347.97 371.68	40 100 60
4	12.0	12.3 12.5 12.4	7.31 7.26 7.26	1836.27 1270.90 1406.59	764.09 562.75 623.91	0 0 0
		12.5	7.38	967.85	364.01	80

APPENDIX 5A CONTINUED
RAINBOW TROUT JUVENILES
1 HOUR BIOASSAY AT pH 8.0

Design H ₂ S Level	Design Temp °C	Observed Temp °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	% Survival
0 (Control)	4.0	7.8	7.68	<5	<1.31	100
						100
						100
	12.0	13.0	7.86	<5	<0.82	100
						100
						100
	20.0	20.2	7.70	<5	<0.95	100
						100
						100
1	8.0	8.5	8.03	492.94	65.66	100
		8.5	7.99	393.43	56.82	100
		8.6	8.06	479.37	60.04	100
	16.0	16.5	8.13	370.84	32.30	100
		16.5	8.15	361.77	30.21	100
		16.4	8.09	438.66	41.65	100
2	4.0	6.9	7.91	976.90	172.91	100
		6.8	8.01	886.44	129.75	100
		6.8	7.98	976.90	151.63	100
	12.0	13.1	8.02	981.41	117.18	100
		13.2	8.05	940.71	105.38	100
		12.7	8.04	1008.56	116.88	100
	20.0	20.1	8.05	872.87	82.49	100
		20.1	8.04	963.33	92.96	100
		20.1	8.03	791.45	77.98	100
	16.0	8.7	8.02	1225.66	165.81	80
		9.0	8.00	1198.52	167.20	60
		8.7	8.04	1266.37	164.61	80
		8.8	7.96	1605.59	243.74	80
		9.1	8.04	1573.94	202.13	100
		8.9	7.97	1329.69	197.38	60
		16.4	8.01	1334.21	149.43	100
		16.4	8.04	1430.55	150.65	80
		16.5	7.93	1415.63	185.95	100
3	16.0	16.4	8.01	1836.27	205.66	20
		16.5	8.03	2012.66	215.83	0
		16.5	8.08	2012.66	194.63	20
4	12.0	11.8	7.89	2211.68	353.76	20
		12.2	7.93	2704.68	480.23	0
		12.0	7.83	2555.42	456.07	0

APPENDIX 5A CONTINUED
RAINBOW TROUT JUVENILES
1 HOUR BIOASSAY AT pH 8.5

Design H ₂ S Level	Design Temp °C	Observed Temp °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	% Survival
0 (Control)	4.0	9.0	8.24	<5	<0.43	100 100 100
	12.0	11.7	8.41	<5	<0.27	100 100 100
	20.0	19.5	8.12	<5	<0.41	100 100 100
1	8.0	8.5	8.48	1525.66	79.03	100
		8.2	8.48	1272.38	66.57	100
		8.2	8.44	1417.11	80.89	100
	16.0	15.7	8.59	1290.47	42.22	100
		15.7	8.57	1417.11	48.48	100
		15.6	8.57	1453.30	49.85	100
2	4.0	6.8	8.31	1905.60	150.81	100
		6.5	8.35	2050.33	150.59	100
		6.7	8.36	2176.98	155.42	100
	12.0	10.9	8.55	2176.98	89.50	100
		11.0	8.49	2213.16	103.50	80
		10.5	8.48	2267.44	110.10	80
	20.0	19.3	8.41	1869.41	83.05	100
		19.3	8.38	1760.86	83.56	100
		19.3	8.49	1616.12	60.17	100
3	8.0	8.1	8.31	2599.12	196.87	0
		8.5	8.33	2617.21	187.48	60
		8.2	8.30	2575.00	198.58	20
	16.0	16.0	8.30	2731.80	167.71	40
		15.8	8.33	2490.57	144.03	60
		16.0	8.31	2683.55	161.22	40
4	12.0	10.4	8.40	4981.16	288.85	20
		10.6	8.41	4548.46	256.50	20
		10.5	8.40	4295.17	248.30	60
		13.3	8.32	5952.10	376.60	0
		13.4	8.32	5941.54	374.87	0
		13.3	8.32	6110.40	386.61	0

APPENDIX 5B - ANALYSIS OF RAINBOW TROUT JUVENILE BIOASSAY DATA.
TOTAL H₂S AT PH 7.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - TRANSFORMED DATA

SOURCE	SS	D.F.	MSS	APPROX. F	MLR
REGRESSION	24207.6865	5	4841.54	22.86	.602713-12*
LINEAR	16119.9274	2	8059.96	38.06	.127730-09*
X1 LINEAR	207.4017	1	207.40	.98	.545677+00
X2 LINEAR	16117.0483	1	16117.05	76.11	.128016-09*
QUADRATIC	4228.5228	2	2114.26	9.98	.817685-04*
X1 QUAD.	47.1606	1	47.16	.22	.869668+00
X2 QUAD.	3962.3430	1	3962.34	18.71	.129676-03*
X1 * X2	163.0764	1	163.08	.77	.619976+00
TRANSFORM	161.4641	1	161.46	.00	.622872+00
RESIDUAL	5717.7222	27	211.77		
TOTAL	29925.4087	32			

RELATIVE LIKELIHOOD OF NO TRANSFORMATION VS. ML ESTIMATES = .62287+00
REGRESSION:

$$Y^{0.8037} = 44.9487 - 0.773044X_1 + 0.0143664X_2 + 0.0309098X_1^2 - 0.0000176322X_2^2 - 0.00076599X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean residual for Model (34 Data Points) = 7.33%

* Indicates significance at 0.05 probability level

APPENDIX 5C - ANALYSIS OF RAINBOW TROUT JUVENILE BIOASSAY DATA.
TOTAL H₂S AT PH 8.0 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - TRANSFORMED DATA

SOURCE	SS	C.F.	MSS	APPROX. F	MLR
REGRESSION	34890.8867	5	6978.18	42.62	.574190-17*
LINEAR	21688.8977	2	10842.95	66.22	.137587-13*
X1 LINEAR	249.9163	1	249.92	1.53	.403111+00
X2 LINEAR	21611.5488	1	21611.55	131.98	.145209-13*
QUADRATIC	9215.7167	2	4607.86	28.14	.254763-08*
X1 QUAD.	13.4883	1	13.49	.08	.951105+00
X2 QUAD.	8923.2031	1	8923.20	54.49	.379543-08*
X1 * X2	575.6251	1	575.63	3.52	.131015+00
TRANSFORM	1402.8804	1	1402.88	.00	.979474-02*
RESIDUAL	5239.9512	32	163.75		
TOTAL	40130.8379	38			

RELATIVE LIKELIHOOD OF NO TRANSFORMATION VS. ML ESTIMATES = .97947402

REGRESSION:

$$Y^{0.6476} = 16.3429 + 0.355915X_1 + 0.00765227X_2 - 0.00756427X_1^2 - 0.00000460657X_2^2 - 0.000282405X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean residual for Model (39 Data Points) = 9.06%

* Indicates significance at 0.05 probability level.

APPENDIX 5D - ANALYSIS OF RAINBOW TROUT JUVENILE BIOASSAY DATA. TOTAL H₂S AT PH 8.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - TRANSFORMED DATA

SOURCE	SS	D.F.	MSS	APPROX. F	MLR
REGRESSION	24797.5764	5	4959.52	15.37	.294570-10 *
LINEAR	17413.0366	2	8706.52	26.98	.260132-08 *
X1 LINEAR	13.6048	1	13.60	.04	.972304+00
X2 LINEAR	14639.5298	1	14639.53	45.37	.196127-07 *
QUADRATIC	311.6251	2	155.81	.48	.531212+00
X1 QUAD.	17.1303	1	17.13	.05	.965260+00
X2 QUAD.	277.6574	1	277.66	.86	.568525+00
X1, X2	67.2468	1	67.25	.21	.870747+00
TRANSFORM	4395.2632	3	1465.79	.00	.641141-03 *
RESIDUAL	8712.1616	27	322.67		
TOTAL	33509.7378	35			

RELATIVE LIKELIHOOD OF NO TRANSFORMATION VS. ML ESTIMATES = .64114-03
REGRESSION:

$$Y^{0.586} = 64.6022 - 73.3564X_1^{0.148} + 0.00000156602X_2^{1.926} + 27.1168X_1^{0.236} \\ + (0.222120) \cdot 10^{-13} X_2^{3.832} - 0.00000188788X_1^{0.148} X_2^{1.926}$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean residual for Model (36 Data Points) = 12.89%

* Indicates significance at 0.05 probability level

APPENDIX 5E - ANALYSIS OF RAINBOW TROUT JUVENILE BIOASSAY DATA.
AQUEOUS H₂S AT PH 7.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - TRANSFORMED DATA

SOURCE	SS	D.F.	MSE	APPROX. F	MLR
REGRESSION	25941.3782	5	5192.28	35.13	.131442-14*
LINEAR	17409.5332	2	8704.77	54.89	.398904-12*
X1 LINEAR	470.4839	1	420.45	2.84	.182165+00
X2 LINEAR	17390.7446	1	17390.74	117.56	.404905-12*
QUADRATIC	4449.9618	2	2224.98	15.05	.294802-05*
X1 QUAD.	8.4576	1	8.46	.06	.964650+00
X2 QUAD.	4332.6055	1	4332.61	29.31	.374025-05*
X1 * X2	378.5573	1	378.54	2.56	.214433+00
TRANSFORM	241.9109	1	241.91	.00	.367708+00
RESIDUAL	3990.7946	27	147.81		
TOTAL	29952.1768	33			

RELATIVE LIKELIHOOD OF NO TRANSFORMATION VS. ML ESTIMATES = .36771+00

REGRESSION:

$$Y^{0.799} = 40.2266 - 0.217977X_1 + 0.0476669X_2 + 0.0129664X_1^2 - 0.000100834X_2^2 - 0.00287646 X_1 X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean residual for Model (34 Data Points) = 6.30%

* Indicates significance at 0.05 probability level.

APPENDIX 5F - ANALYSIS OF RAINBOW TROUT JUVENILE BIOASSAY DATA.
AQUEOUS H₂S AT PH 8.0 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA				
SOURCE	SS	D.F.	MSS	F
REGRESSION	30031.0742	5	6006.21	17.38 *
INTERCEPT	23283.5444	1	11641.77	33.69 *
X1: LINEAR	2125.3166	1	2125.32	6.15 *
X2: LINEAR	23282.6868	1	23282.69	67.37 *
QUADRATIC	892.9889	2	446.49	1.29
X1 QUAD.	20.4354	1	20.44	.06
X2 QUAD.	820.5654	1	820.57	2.37
X1 X2	1760.0815	1	1760.08	5.09 *
RESIDUAL	11404.8213	33		
TOTAL	41435.8955	38		

REGRESSION:

$$Y = 102.246 - 0.529734X_1 + 0.147589X_2 + 0.0516529X_1^2 - 0.000238473X_2^2 - 0.0225387X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean residual for Model (39 Data Points) = 12.35%

* Indicates significance at 0.05 probability level

APPENDIX 5G - ANALYSIS OF RAINBOW TROUT JUVENILE BIOASSAY DATA. AQUEOUS H₂S AT PH 8.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - TRANSFORMED DATA

SOURCE	SS	D.F.	MSS	APPROX. F	MLR
REGRESSION	28251.5511	1	5651.12	25.69	.184716-13*
LINEAR	19992.2384	1	9996.17	45.79	.270500-11*
X1 LINEAR	64.3110	1	64.31	.29	.822557+00
X2 LINEAR	15994.8682	1	15994.87	73.27	.553881-10*
QUADRATIC	1182.9398	2	591.47	2.71	.371685-01*
X1 QUAD.	378.1555	1	378.16	1.73	.326501+00
X2 QUAD.	570.7357	1	570.74	2.61	.189441+00
X1 . X2	2.8516	1	2.85	.01	.991331+00
TRANSFORM	3594.0335	3	1198.28	.00	.189487-03*
RESIDUAL	5894.0552	27	218.30		
TOTAL	34149.6460	35			

RELATIVE LIKELIHOOD OF NO TRANSFORMATION VS. ML ESTIMATES = .18949-03

** REGRESSION:

$$Y^{0.6509} = 1552.32 - 2528.12X_1^{0.074} - 0.00111651X_2^{1.852} + 1042.59X_1^{0.148} + 0.000000004488X_2^{3.7} + 0.000433326X_1^{0.074}X_2^{1.852}$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean residual for Model (36 Data Points) = 9.39%

* Indicates significance at 0.05 probability level

** This model should not be used for estimation of effects at H₂S aqueous levels above 400 ppb, as substantial errors will occur at higher concentrations.

APPENDIX 6A
LAKE WHITEFISH SAC-FRY
1 HOUR BIOASSAY AT pH 7.5

Design H ₂ S Level	Design Temp °C	Observed Temp °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	% Survival
0 (Control)	4.0	4.5	7.62	0	0	100
						100
						100
	10.0	9.9	7.62	0	0	100
						100
						100
	16.0	15.9	7.68	0	0	100
						100
						100
1	7.0	6.2	7.49	244.97	90.01	100
		6.1	7.48	99.72	37.26	100
		6.1	7.45	138.89	54.17	100
	13.0	12.6	7.56	104.62	29.73	100
		12.5	7.59	124.20	33.66	100
		12.5	7.59	119.31	32.22	100
2	4.0	4.0	7.61	241.70	78.51	100
		3.9	7.56	256.39	90.13	100
		4.0	7.59	275.98	92.46	100
	10.0	9.7	7.51	236.81	77.79	100
		9.9	7.53	222.12	70.40	100
		9.6	7.50	251.50	84.08	100
	16.0	15.7	7.62	236.81	56.82	100
		15.7	7.62	231.91	55.64	100
		15.7	7.59	261.29	66.04	100
		15.8	7.58	300.46	77.10	100
		15.8	7.62	284.14	68.03	100
		15.8	7.62	185.77	68.42	90
	7.0	6.0	7.51	388.58	139.33	100
		6.0	7.49	413.06	152.51	100
		6.2	7.45	320.04	124.52	100
3	13.0	12.5	7.59	339.62	92.03	100
		12.5	7.60	339.62	90.49	100
		12.5	7.55	354.31	102.59	90
	7.0	7.1	7.50	450.60	159.65	100
		7.1	7.52	579.52	199.26	100
		7.1	7.52	378.79	130.24	100
	13.0	12.5	7.56	390.21	111.15	100
		12.5	7.60	382.05	101.80	100
		12.5	7.57	390.21	109.32	100
4	10.0	9.8	7.43	1214.45	448.77	0
		9.8	7.54	687.23	214.90	30
		9.8	7.59	780.26	225.11	40
		10.0	7.46	1295.23	455.97	0
		9.8	7.47	817.79	284.87	10
		10.0	7.49	980.99	330.06	20

APPENDIX 6A CONTINUED
LAKE WHITEFISH SAC-FRY
1 HOUR BIOASSAY AT pH 8.0

Design H ₂ S Level	Design Temp °C	Observed Temp °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	% Survival
0 (Control)	4.0	4.6	8.10	<10	<1.32	100 100 100
		10.0	8.13	<10	<1.04	100 100 100
	10.0	16.8	8.07	<10	<0.98	100 100 100
	16.0					
1	7.0	6.6	8.01	267.99	39.48	100
		6.5	8.05	248.40	33.95	100
		6.5	8.04	326.74	45.51	100
	13.0	12.7	8.07	287.57	31.34	100
		12.5	8.07	326.74	35.81	100
		12.5	8.05	267.99	30.60	100
2	4.0	4.0	8.04	669.45	101.52	100
		4.0	8.02	548.69	86.51	100
		4.1	7.97	656.40	113.53	100
	10.0	9.6	8.01	578.07	77.67	100
		9.8	8.03	512.78	65.81	100
		9.5	8.01	571.54	77.01	100
	16.0	15.8	7.99	702.09	83.12	100
		15.8	8.02	669.45	74.56	100
		15.8	8.03	646.61	70.55	100
3	7.0	6.8	7.97	835.92	132.29	100
		6.7	7.97	796.75	126.49	100
		6.7	7.98	600.91	93.57	100
	13.0	12.5	8.04	672.72	78.38	100
		12.5	8.05	659.66	75.31	100
		12.6	7.99	728.21	93.62	100
	7.0	7.1	7.87	1390.79	263.80	60
		7.1	7.87	1387.52	263.18	40
		7.4	7.91	1234.12	215.14	80
	13.0	12.7	7.98	1025.22	134.11	100
		12.7	7.99	1064.39	136.47	80
		12.7	7.99	920.78	118.05	90
4	10.0	9.7	7.81	1687.81	332.31	0
		9.7	7.84	1514.82	282.05	20
		9.7	7.81	1834.69	361.23	0

APPENDIX 6A CONTINUED
LAKE WHITEFISH SAC-FRY
1 HOUR BIOASSAY AT pH 8.5

Design H ₂ S Level	Design Temp °C	Observed Temp °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	% Survival
0 (Control)	4.0	4.7	8.40	0	0	100 100 100
		9.5	8.55	0	0	100 100 100
		15.9	8.57	0	0	100 100 100
	10.0	7.2 6.9 6.9	8.47 8.43 8.41	508.32 357.91 430.96	28.13 21.83 27.44	100 100 100
		12.6 12.7 12.6	8.57 8.51 8.53	379.40 469.64 443.84	14.17 18.06 18.11	100 100 100
		4.0 4.2 4.2	8.34 8.29 8.24	895.08 886.48 1075.56	73.59 80.36 108.20	100 100 100
2	4.0	4.5 4.7 4.7	8.30 8.31 8.26	1088.48 1062.68 1281.84	95.54 90.66 121.43	100 100 100
		9.6 9.7 9.4	8.42 8.43 8.44	856.40 779.05 753.28	48.77 43.27 41.33	100 100 100
		15.7 15.8 15.7	8.45 8.47 8.47	903.67 895.08 882.20	40.31 38.11 37.60	90 100 100
	10.0	15.8 15.9 15.9	8.39 8.38 8.44	122.83 1359.20 1178.72	62.01 70.31 53.47	100 90 80
		7.6 7.6 8.2	8.15 8.16 8.21	2403.45 2287.43 1681.48	258.68 241.18 156.75	0 0 0
		13.0 13.0 13.0	8.36 8.34 8.41	1733.05 1823.32 1565.48	101.43 111.43 82.18	0 20 60
	7.0	7.4 7.4 7.6	8.20 8.28 8.32	1900.66 1758.85 1221.69	185.70 145.32 92.11	40 50 100
		12.9 13.0 12.8	8.38 8.38 8.43	1363.49 1526.79 1165.81	76.63 85.56 58.92	90 80 80
		9.5 9.5 9.5	8.20 8.19 8.20	2132.72 1939.32 2222.96	194.85 180.93 203.10	0 10 0
	13.0	7.0	8.20	1900.66	185.70	40
		7.4	8.28	1758.85	145.32	50
		7.6	8.32	1221.69	92.11	100

APPENDIX 6B - ANALYSIS OF LAKE WHITEFISH SAC-FRY BIOASSAY DATA.
TOTAL H₂S AT PH 7.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA				F
	SS	D.F.	MSS	
SOURCE				
REGRESSION	31751.9460	5	6350.39	45.53 *
LINEAR	21508.7224	2	10754.36	77.11 *
X1 LINEAR	85.5164	1	85.52	.61
X2 LINEAR	21371.7378	1	21371.74	153.24 *
QUADRATIC	3691.1655	2	1845.58	13.23 *
X1 QUAD.	58.6954	1	58.70	.42
X2 QUAD.	3646.2451	1	3646.25	26.14 *
X1 * X2	69.6613	1	69.66	.50
RESIDUAL	5439.1641	39	139.47	
TOTAL	37191.1099	44		

REGRESSION:

$$Y = 108.843 - 1.61834X_1 + 0.0187425X_2 + 0.0917323X_1^2 - 0.0000706952X_2^2 - 0.00222894X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean residual for Model (45 Data Points) = 6.63%

* Indicates significance at 0.05 probability level.

APPENDIX 6C - ANALYSIS OF LAKE WHITEFISH SAC-FRY BIOASSAY DATA.
TOTAL H₂S AT PH 8.0 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	26287.3018	5	5257.46	117.89 *
LINEAR	14001.7432	2	7000.87	156.98 *
X1 LINEAR	2.4851	1	2.49	.06
X2 LINEAR	13542.3081	1	13542.31	303.67 *
QUADRATIC	8840.6583	2	4420.33	99.12 *
X1 QUAD.	16.4033	1	16.40	.37
X2 QUAD.	8769.0447	1	8769.04	196.63 *
X1 * X2	.0070	1	.01	.00
RESIDUAL	1471.6716	13	44.60	
TOTAL	27758.9734	18		

REGRESSION:

$$Y = 100.755 - 0.936954X_1 + 0.0410636X_2 + 0.0489061X_1^2 - 0.0000537301X_2^2 + 0.00000970159X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean residual for Model (39 Data Points) = 4.31%

* Indicates significance at 0.05 probability level.

APPENDIX 6D - ANALYSIS OF LAKE WHITEFISH SAC-FRY BIOASSAY DATA.
TOTAL H₂S AT PH 8.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE -- TRANSFORMED DATA					
SOURCE	SS	D.F.	MSS	APPROX. F	MLR
REGRESSION	35407.6675	5	7081.53	44.70	.142136-18*
LINEAR	21395.7290	2	10697.86	67.52	.153700-14*
X ₁ LINEAR	82.7156	1	82.72	.52	.735637+00
X ₂ LINEAR	21388.8452	1	21388.85	135.00	.154571-14*
QUADRATIC	9816.0669	2	4908.03	30.98	.354365-09*
X ₁ QUAD.	356.1123	1	356.11	2.25	.274451+00
X ₂ QUAD.	7803.6132	1	7803.61	49.25	.754184-08*
X ₁ * X ₂	90.6131	1	90.61	.57	.714542+00
TRANSFORM	1716.0229	1	1716.02	.00	.354392-02*
RESIDUAL	6020.5913	38	158.44		
TOTAL	41428.2588	44			
RELATIVE LIKELIHOOD OF NO TRANSFORMATION VS. ML ESTIMATES = .35439-02					
REGRESSION:					

$$Y^{0.6698} = 25.8064 - 1.10162X_1 + 0.0082432X_2 + 0.0573235X_1^2 - 0.00000715478X_2^2 - 0.000163205X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean residual for Model (45 Data Points) = 8.81%

* Indicates significance at 0.05 probability level.

APPENDIX 6E - ANALYSIS OF LAKE WHITEFISH SAC-FRY BIOASSAY DATA.
AQUEOUS H₂S AT PH 7.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MS	F
REGRESSION	31016.9739	5	6203.39	39.18 *
LINEAR	22021.9946	2	11011.00	69.55 *
X1 LINEAR	599.8377	1	599.84	3.79
X2 LINEAR	21609.9858	1	21509.99	136.50 *
QUADRATIC	1412.2001	2	706.10	4.46 *
X1 QUAD.	32.2226	1	32.22	.20
X2 QUAD.	1411.2432	1	1411.24	8.91 *
X1 * X2	482.5624	1	482.56	3.05
RESIDUAL	6174.1352	39	158.31	
TOTAL	37191.1039	44		

REGRESSION:

$$Y = 105.295 - 0.786762X_1 + 0.0995853X_2 + 0.0693413X_1^2 - 0.000366809X_2^2 - 0.0194182X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean residual for Model (45 Data Points) = 8.29%

* Indicates significance at 0.05 probability level.

APPENDIX 6F - ANALYSIS OF LAKE WHITEFISH SAC-FRY BIOASSAY DATA.
 AQUEOUS H₂S AT PH 8.0 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	26682.2402	5	5336.45	163.55 *
LINEAR	17397.4297	2	8698.71	266.60 *
X1 LINEAR	103.7200	1	103.72	3.18
X2 LINEAR	15803.7540	1	15803.75	484.35 *
QUADRATIC	4984.2797	2	2492.14	76.38 *
X1 QUAD.	1.9142	1	1.93	.06
X2 QUAD.	4982.5052	1	4982.51	152.71 *
X1 * X2	85.9057	1	85.91	2.63
RESIDUAL	1076.7332	33	32.63	
TOTAL	27758.9734	38		

REGRESSION:

$$Y = 99.4848 - 0.188438X_1 + 0.169001X_2 + 0.0171204X_1^2 - 0.00112955X_2^2 - 0.00741772X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean residual for Model (39 Data Points) = 3.33%

* Indicates significance at 0.05 probability level.

APPENDIX 6G - ANALYSIS OF LAKE WHITEFISH SAC-FRY BIOASSAY DATA
 AQUEOUS H₂S AT PH 8.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA			
SOURCE	SS	D.F.	F
REGRESSION	51450.9404	5	10290.19
LINEAR	35699.3867	2	17849.69
X1 LINEAR	4852.9152	1	4852.92
X2 LINEAR	35038.7417	1	35038.74
QUADRATIC	3251.9852	2	1625.99
X1 QUAD.	485.2359	1	485.24
X2 QUAD.	2041.4668	1	2041.47
X1 * X2	3263.3437	1	3263.34
RESIDUAL	9580.1660	39	245.65
TOTAL	61031.1064	44	

REGRESSION:

$$Y = 119.010 - 4.50944X_1 + 0.338088X_2 + 0.273373X_1^2 - 0.00137586X_2^2 - 0.0583071X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean Residual for Model (45 Data Points) = 9.55%

* Indicates significance at 0.05 probability level.

APPENDIX 7A
ROUND WHITEFISH SAC-FRY
2 HOUR BIOASSAY AT pH 7.5

Design H ₂ S Level	Design Temp °C	Observed Temp °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	Survival
0 (Control)	4.0	4.8	7.41	<5	<2.12	100
						100
						100
	10.0	10.5	7.45	<5	<1.77	100
						100
						100
1	16.0	16.8	7.63	<5	<1.15	100
						100
						100
	7.0	7.2	7.33	115.01	51.42	100
		7.0	7.33	94.92	42.61	100
		7.0	7.29	94.92	44.78	100
2	13.0	13.0	7.31	117.52	48.29	100
		12.7	7.30	102.46	42.90	100
		12.8	7.31	102.46	42.25	100
	4.0	4.2	7.36	222.98	102.35	100
		4.4	7.35	273.19	126.40	100
		4.3	7.33	210.42	99.99	100
3	10.0	10.3	7.43	255.61	93.46	100
		10.4	7.45	278.21	98.56	100
		10.4	7.36	222.98	89.86	100
	16.0	16.6	7.52	268.17	74.91	100
		16.7	7.57	222.98	57.14	100
		16.7	7.56	250.59	65.32	100
4	7.0	7.1	7.34	338.47	149.71	90
		7.1	7.33	398.73	178.63	80
		7.1	7.31	396.22	182.03	90
	13.0	12.8	7.31	373.62	154.07	70
		13.0	7.33	381.16	152.39	40
		12.9	7.31	416.31	171.37	20
4	10.0	10.5	7.39	609.69	235.14	0
		10.5	7.36	654.84	263.37	0
		10.5	7.38	772.84	302.31	0

APPENDIX 7A CONTINUED
 ROUND WHITEFISH SAC-FRY
 1 HOUR BIOASSAY AT pH 8.0

Design H ₂ S Level	Design Temp °C	Observed Temp °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	% Survival
0 (Control)	4.0	4.9	8.08	<5	0.68	100
						100
						100
	10.0	9.6	8.17	<5	0.48	100
						100
						100
	16.0	16.5	8.18	<5	0.39	80
						100
						100
1	7.0	7.3	8.13	146.31	16.55	100
		7.0	8.06	139.12	18.32	100
		6.8	8.05	139.12	18.81	100
	13.0	12.8	8.14	182.25	17.14	100
		12.6	8.16	167.87	15.22	100
		12.8	8.14	167.87	15.78	100
2	4.0	4.7	8.08	261.30	35.73	100
		4.6	8.03	278.08	42.11	100
		4.7	8.04	282.87	41.86	100
	10.0	10.0	8.12	251.72	26.74	100
		10.0	8.13	369.11	38.41	100
		9.6	8.07	294.84	35.11	100
	16.0	16.4	8.16	316.40	25.94	100
		16.4	8.17	290.05	23.28	100
		16.3	8.16	282.87	23.25	100
3	7.0	7.2	8.05	589.52	78.71	50
		7.2	8.04	642.22	87.46	10
		7.0	7.98	668.58	103.12	10
	13.0	12.8	8.02	644.62	77.59	0
		12.9	8.00	769.41	95.54	0
		12.9	8.05	709.31	80.10	0
4	10.0	9.9	7.98	781.18	110.43	0
		9.8	7.98	790.76	112.11	0
		9.7	8.02	685.35	89.99	0

APPENDIX 7A CONTINUED
ROUND WHITEFISH SAC-FRY
1 HOUR BIOASSAY AT pH 8.5

Design H ₂ S Level	Design Temp °C	Observed Temp °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	Survival
0 (Control)	4.0	4.7	8.50	< 5	< 0.28	100 100 100
	10.0	10.0	8.65	< 5	< 0.17	100 100 100
	16.0	16.5	8.63	< 5	< 0.15	100 100 100
1	7.0	6.5	8.62	208.28	8.50	100
		6.5	8.65	184.25	7.04	100
		6.5	8.65	197.35	7.54	100
	13.0	12.9	8.70	259.90	7.20	100
		12.8	8.68	267.30	7.77	100
		13.0	8.71	240.17	6.49	100
2	4.0	4.5	8.50	533.78	30.55	100
		4.3	8.47	578.19	35.59	100
		4.5	8.48	511.57	30.58	100
	10.0	9.7	8.61	578.19	21.63	100
		9.9	8.59	627.54	24.38	100
		10.1	8.51	592.99	27.30	100
	16.0	16.4	8.57	541.18	18.17	100
		16.5	8.52	504.17	18.86	100
		16.4	8.58	526.38	17.28	100
3	7.0	6.8	8.51	763.24	39.25	100
		6.7	8.47	808.44	45.53	100
		6.7	8.53	817.13	40.37	100
	13.0	12.9	8.58	874.27	31.66	90
		13.0	8.55	1022.31	39.45	60
		13.0	8.58	1054.39	38.07	0
4	10.0	10.3	8.39	1540.73	91.59	0
		10.4	8.43	1442.04	78.34	0
		10.5	8.33	2017.76	135.68	0

APPENDIX 7B - ANALYSIS OF ROUND WHITEFISH SAC-FRY BIOASSAY DATA.
TOTAL H₂S AT PH 7.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	28563.8743	5	5712.77	30.52 *
LINEAR	18937.2900	2	9468.65	50.58 *
X1 LINEAR	566.5601	1	566.56	3.03
X2 LINEAR	18369.3596	1	18369.36	98.13 *
QUADRATIC	4428.5060	2	2214.25	11.83 *
X1 QUAD.	190.4931	1	190.49	1.02
X2 QUAD.	4084.7300	1	4084.73	21.39 *
X1 * X2	603.0928	1	603.09	3.22
RESIDUAL	5054.3062	27	187.20	
TOTAL	33618.1802	32		

REGRESSION:

$$Y = 110.739 - 2.84827X_1 + 0.0958622X_2 + 0.162476X_1^2 - 0.000224574X_2^2 - 0.00799555X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean Residual for Model (33 Data Points) = 8.32%

* Indicates significance at 0.05 probability level.

APPENDIX 7C - ANALYSIS OF ROUND WHITEFISH SAC-FRY BIOASSAY DATA.
TOTAL H₂S AT PH 8.0 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - TRANSFORMED DATA

SOURCE	SS	D.F.	MSS	APPROX. F	MLP
REGRESSION	20590.6611	5	4118.12	121.15	.386544-23*
LINEAR	15605.8295	2	7802.91	229.55	.306775-21*
X1 LINEAR	241.9650	1	241.96	7.12	.137633-01*
X2 LINEAR	13014.3437	1	13014.34	382.87	.521852-20*
QUADRATIC	557.7700	2	278.88	8.20	.184764-03*
X1 QUAD.	.1993	1	.20	.01	.995978+00
X2 QUAD.	556.5397	1	556.54	16.37	.187516-03*
X1 * X2	257.1820	1	257.18	7.57	.108735-01*
TRANSFORM	2276.6541	3	758.88	.00	.282600-09*
RESIDUAL	815.8069	24	33.99		
TOTAL	21406.4680	32			

RELATIVE LIKELIHOOD OF NO TRANSFORMATION VS. ML ESTIMATES = .28260-09

REGRESSION:

$$Y^{0.4893} = 11.4545 - 2.05119X_1^{0.222} + (0.535761)10^{-8} X_2^{3.185} + 0.597908X_1^{0.444} + (0.328914)10^{-17} X_2^{6.37}$$

$$- (0.981332)10^{-8} X_1 X_2^{0.222}$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean Residual for Model (33 Data Points) = 6.87%

* Indicates significance at 0.05 probability level.

APPENDIX 7D - ANALYSIS OF ROUND WHITEFISH SAC-FRY BIOASSAY DATA.
TOTAL H₂S AT PH 8.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNREPLICATED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	27919.0503	5	5583.81	19.72 *
LINEAR	18319.1616	2	9159.58	32.35 *
X1 LINEAR	824.6353	1	824.64	2.91
X2 LINEAR	16884.9465	1	16884.95	59.64 *
QUADRATIC	3546.6087	2	1773.30	6.26 *
X1 QUAD.	86.9539	1	86.95	.31
X2 QUAD.	3366.5565	1	3366.56	11.89 *
X1 * X2	883.4969	1	883.50	3.12
RESIDUAL	7644.5845	27	283.13	
TOTAL	35563.6348	32		

REGRESSION:

$$Y = 106.362 - 1.77074X_1 + 0.0422515X_2 + 0.119577X_1^2 - 0.0000314112X_2^2 - 0.00425278X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean Residual for Model (33 Data Points) = 9.81%

* Indicates significance at 0.05 probability level.

APPENDIX 7E - ANALYSIS OF ROUND WHITEFISH SAC-FRY BIOASSAY DATA
AQUEOUS H₂S AT pH 7.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	29694.9612	5	5939.79	40.92 *
LINEAR	19950.9812	2	9975.49	68.72 *
X1 LINEAR	1973.3825	1	1973.38	13.59 *
X2 LINEAR	19605.9143	1	19605.91	135.07 *
QUADRATIC	3187.7252	2	1593.86	10.98 *
X1 QUAD.	1.8842	1	1.88	.01
X2 QUAD.	3176.5795	1	3176.58	21.88 *
X1 * X2	1912.3847	1	1912.38	13.17 *
RESIDUAL	3919.2192	27	145.16	
TOTAL	33610.1802	32		

REGRESSION:

$$Y = 94.1932 + 0.590998X_1 + 0.380484X_2 + 0.169702X_1^2 - 0.00134727X_2^2 - 0.0368379X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean Residual for Model (33 Data Points) = 8.11%

* Indicates significance at 0.05 probability level.

APPENDIX 7F - ANALYSIS OF ROUND WHITEFISH SAC-FRY BIOASSAY DATA.
 AQUEOUS H₂S AT PH 8.0 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSE	F
REGRESSION	52192.7368	5	10438.55	56.22 *
LINEAR	19942.8604	2	9971.43	107.61 *
X1 LINEAR	1422.1987	1	1422.20	7.56 *
X2 LINEAR	19959.7773	1	19959.78	215.21 *
QUADRATIC	3016.3492	2	1508.17	8.12 *
X1 QUAD.	.1106	1	.11	.00
X2 QUAD.	2991.5250	1	2991.53	16.11 *
X1 * X2	926.1128	1	926.11	4.79 *
RESIDUAL	5013.3188	27	185.68	
TOTAL	57206.0557	32		

REGRESSION:

$$Y = 102.368 - 0.0227652X_1 + 0.455971X_2 + 0.00455999X_1^2 - 0.00934829X_2^2 - 0.0544499X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean Residual for Model (33 Data Points) = 8.79%

* Indicates significance at 0.05 probability level.

APPENDIX 7G - ANALYSIS OF ROUND WHITEFISH SAC-FRY BIOASSAY DATA.
 AQUEOUS H₂S AT PH 8.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSE	F
REGRESSION	26571.7317	5	5314.35	15.96 *
LINEAR	20097.9440	2	10048.99	30.17 *
X1 LINEAR	2940.4768	1	2940.48	8.83 *
X2 LINEAR	18471.3247	1	18471.32	55.46 *
QUADRATIC	88.6803	2	44.34	.13
X1 QUAD.	78.7552	1	78.76	.24
X2 QUAD.	6.1465	1	6.15	.02
X1 * X2	2518.6246	1	2518.62	7.56 *
RESIDUAL	18991.9033	27	699.70	
TOTAL	35563.6348	32		

REGRESSION:

$$Y = 107.403 - 1.43375X_1 + 0.707106X_2 + 0.115378X_1^2 + 0.000333845X_2^2 - 0.158651X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean Residual for Model (33 Data Points) = 11.73%

* Indicates significance at 0.05 probability level.

APPENDIX 8A
ROCK BASS SAC-FRY
1 HOUR BIOASSAY AT pH 7.5

Design H ₂ S Level	Design Temp °C	Observed Temp °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	Survival
0 (Control)	12.0	12.0	7.59	<17.26	<4.73	100 100 80
	20.0	20.3	7.54	<17.26	<4.34	100 80 100
	28.0	26.7	7.66	<17.26	<3.11	100 100 100
1	16.0	15.6 15.3 15.5	7.53 7.44 7.46	414.66 319.08 454.90	116.23 103.96 143.09	100 100 100
	24.0	23.9 23.9 23.9	7.53 7.50 7.52	490.11 364.35 334.17	117.56 92.07 81.57	100 100 90
2	12.0	11.5 11.5 11.5	7.44 7.49 7.49	827.14 646.05 766.78	290.65 210.37 249.68	70 50 90
	20.0	20.4 20.5 20.5	7.37 7.95 7.35	761.75 696.35 776.84	252.50 202.97 265.06	70 90 100
	28.0	27.5 27.3 27.5	7.53 7.54 7.53	696.35 711.44 681.26	157.19 158.27 153.78	90 100 100
3	16.0	15.7 15.7 15.5	7.43 7.45 7.41	1269.81 1491.14 1154.11	416.98 474.64 392.26	50 20 40
	24.0	23.8 23.8 23.8	7.53 7.45 7.42	1420.72 1269.81 1450.90	341.39 349.84 420.04	0 0 0
4	20.0	20.4 20.5 20.5	7.34 7.31 7.36	1647.08 1647.08 1868.42	571.47 596.57 627.87	10 0 0
		20.4 15.8	7.29 7.40	2698.42 1088.72	1007.81 373.61	0 90

APPENDIX 8A CONTINUED
 ROCK BASS SAC-FRY
1 HOUR BIOASSAY AT pH 7.5 (TEST #2)

Design H ₂ S Level	Design Temp °C	Observed Temp °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	% Survival
0 (Control)	10.0	10.5	7.67	<32.35	<8.02	100
	18.0	17.9	7.66	<17.26	<3.68	100
	26.0	25.8	7.74	<32.35	<5.08	100
						100
1	14.0	13.8	7.51	379.44	114.03	100
		13.7	7.50	349.26	106.88	100
	22.0	13.7	7.53	334.17	97.42	100
		22.2	7.50	243.63	63.41	100
		22.3	7.52	303.99	76.31	100
		22.2	7.51	228.53	58.47	100
2	10.0	9.8	7.51	786.90	257.89	100
		9.8	7.51	656.11	215.03	100
	18.0	9.9	7.53	620.90	196.79	90
		17.8	7.47	580.66	172.05	80
		17.7	7.59	545.44	132.31	100
	26.0	17.8	7.54	570.60	150.54	90
		26.4	7.51	575.63	137.07	90
		26.4	7.65	575.63	106.27	100
		26.4	7.59	590.72	121.88	100
3	14.0	13.7	7.42	1068.60	370.23	90
		13.7	7.44	1209.45	406.51	80
		14.0	7.47	801.99	255.79	80
	22.0	22.1	7.50	696.35	181.55	80
		22.2	7.48	560.54	150.91	80
		22.3	7.49	666.17	176.04	90
		22.1	7.54	922.72	224.55	90
		22.1	7.52	973.02	245.14	100
		22.2	7.50	892.54	232.29	90
4	18.0	17.5	7.42	1813.08	584.90	0
		17.5	7.41	1501.20	491.87	0
		17.5	7.44	1667.21	521.20	0

APPENDIX 8A CONTINUED
ROCK BASS SAC-FRY
1 HOUR BIOASSAY AT pH 8.0

Design H ₂ S Level	Design Temp °C	Observed Temp °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	Survival		
0 (Control)	12.0	12.1	8.17	0	0	80 90 70		
	20.0	19.4	8.02	<17.26	<1.76	100 100 100		
	28.0	26.7	8.23	<17.26	<0.96	100 100 100		
	1	16.0	15.2	8.07	842.23	85.92	100	
		24.0	15.1	8.09	560.54	55.00	90	
			15.1	8.08	736.60	73.80	100	
23.8			8.14	756.72	54.52	90		
23.9			8.15	666.17	46.89	100		
23.9			8.18	620.90	40.97	100		
2	12.0		11.5	7.96	1340.23	188.44	60	
	20.0	11.5	8.01	1073.63	136.63	50		
		11.7	7.98	1350.30	181.47	50		
		18.9	8.01	1149.08	121.27	80		
		18.9	8.01	1159.14	122.33	90		
		19.0	8.03	1254.72	126.77	100		
		27.4	7.97	1310.05	125.64	100		
		27.3	7.97	1486.11	142.80	100		
		27.3	7.99	1345.26	123.98	100		
		3	16.0	15.2	7.95	1752.72	228.30	100
			24.0	15.1	7.87	2069.63	316.55	100
				15.4	7.93	1692.36	228.30	100
15.8				7.90	2190.36	310.56	90	
15.7	7.81			2622.97	444.11	60		
15.8	7.90			2205.45	312.70	90		
23.9	8.02			1566.60	145.15	100		
23.9	8.02			1380.48	127.91	100		
23.9	7.98			1531.38	154.20	90		
23.9	7.91			1984.11	230.67	100		
23.8	7.95			1873.45	201.10	75		
23.8	7.90			1979.08	235.28	100		
4	20.0	19.0		7.87	2280.90	318.75	10	
		18.9	7.90	1893.57	244.37	100		
		18.9	7.86	2094.78	292.77	50		
		20.2	7.74	2627.99	460.70	0		
		20.1	7.81	2472.06	379.54	0		
		20.1	7.81	2698.42	413.48	0		

APPENDIX 8A CONTINUED
ROCK BASS SAC-FRY
1 HOUR BIOASSAY AT pH 8.5

Design H ₂ S Level	Design Temp °C	Observed Temp °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	% Survival
0 (Control)	12.0	12.4	9.16	<43.14	<0.43	85.71 100 100
		20.0	8.45	<43.14	<1.65	100 100 100
		28.0	8.64	<23.01	<0.51	100 100 100
	24.0	15.7	8.47	1867.46	79.72	62.5
		15.3	9.23	1330.90	10.35	71.43
		15.2	9.20	1693.08	14.14	42.86
		24.0	8.52	1773.57	55.36	100
		24.0	8.45	1491.87	54.41	100
		24.0	8.49	1411.38	47.10	100
2	12.0	11.6	9.03	2980.84	40.81	28.57
		11.6	9.10	2759.51	32.22	14.29
		11.7	9.16	2920.48	29.65	85.71
	20.0	20.4	8.37	2336.96	110.40	100
		20.4	8.37	2404.03	113.57	100
		20.2	8.84	2739.38	45.49	100
	28.0	27.6	8.17	2900.36	181.22	57.14
		27.5	8.23	2994.25	164.60	42.86
		27.5	8.28	2739.39	135.02	85.71
	24.0	15.7	8.09	3986.90	385.26	0
		15.6	7.96	5720.00	723.08	0
		15.6	9.12	3322.90	32.94	100
		23.9	8.26	3027.79	168.04	100
		24.0	8.36	2437.57	108.47	100
		24.0	8.25	2719.26	153.90	100
3	24.0	20.1	8.55	5290.98	169.05	85.71
		20.3	7.99	3899.71	415.46	0
		20.3	8.01	5560.00	568.40	0
4	20.0	20.1	8.55	5290.98	169.05	85.71
		20.3	7.99	3899.71	415.46	0
		20.3	8.01	5560.00	568.40	0

APPENDIX 8B - ANALYSIS OF ROCK BASS SAC-FRY BIOASSAY DATA.
TOTAL H₂S AT PH 7.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	61982.2974	5	12396.46	34.91 *
LINEAR	48925.3141	2	24463.45	68.90 *
X1 LINEAR	127.9200	1	127.92	.36
X2 LINEAR	48563.8550	1	48563.85	136.77 *
QUADRATIC	2129.2284	2	1064.61	3.00
X1 QUAD.	398.9076	1	398.91	1.12
X2 QUAD.	1600.2400	1	1600.24	4.51 *
X1 * X2	517.3125	1	517.31	1.46
RESIDUAL	23079.6541	65	355.07	
TOTAL	85061.9609	70		

REGRESSION:

$$Y = 125.683 - 3.07193X_1 - 0.00479664X_2 + 0.0970513X_1^2 - 0.0000105744X_2^2 + 0.00127026X_1 X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean residual for model (71 Data Points) = 13.68%

* Indicates significance at 0.05 probability level.

APPENDIX 8C - ANALYSIS OF ROCK BASS SAC-FRY BIOASSAY DATA.
TOTAL H₂S AT PH 8.0 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - TRANSFORMED DATA

SOURCE	SS	D.F.	MS	APPROX. F	MLD
REGRESSION	19598.8718	5	3919.76	8.21	.834741-.07*
LINEAR	10265.1505	2	5132.58	10.75	.426121-.04*
X1 LINEAR	419.2843	1	419.28	.88	.524224+.00
X2 LINEAR	9642.6631	1	9642.66	20.20	.699187-.04*
QUADRATIC	8080.1486	2	4040.07	8.46	.252477-.03*
X1 QUAD.	247.5805	1	247.59	.52	.734244+.00
X2 QUAD.	8077.3847	1	8077.98	16.92	.252240-.03*
X1 * X2	586.3900	1	586.39	1.23	.484115+.00
TRANSFORM	644.3110	1	644.31	1.35	.451754+.00
RESIDUAL	16707.6299	36	464.37		
TOTAL	36306.4917	41			

RELATIVE LIKELIHOOD OF NO TRANSFORMATION VS. ML ESTIMATES = .45175+.00

REGRESSION:

$$Y = 0.8198 = 5.13134 + 2.83646X_1 + 0.0222965X_2 - 0.0508359X_1^2 - 0.00000912728X_2^2 - 0.000444407X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean Residual for Model (42 Data Points) = 16.41%

* Indicates significance at 0.05 probability level.

APPENDIX 8D - ANALYSIS OF ROCK BASS SAC-FRY BIOASSAY DATA.
TOTAL H₂S AT PH 8.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - TRANSFORMED DATA

SOURCE	SS	D.F.	MSS	APPROX. F	MLP
REGRESSION	16463.8738	5	3292.77	6.47	.160002-00 *
LINEAR	13001.0586	2	6750.51	13.27	.912085-00 *
X1 LINEAR	1503.3748	1	1503.32	2.93	.169351-00
X2 LINEAR	10448.0768	1	10448.08	20.33	.587326-00 *
QUADRATIC	1618.0621	3	802.48	1.59	.148454-00
X1 QUAD.	215.4106	1	215.32	.42	.765593-00
X2 QUAD.	1492.6460	1	1492.64	2.93	.171300-00
X1 * X2	527.2215	1	527.22	1.04	.524786-00
TRANSFORM	6599.2334	1	6599.23	12.96	.125560-00 *
RESIDUAL	13230.2308	26	508.86		
TOTAL	29694.1006	32			

RELATIVE LIKELIHOOD OF NO TRANSFORMATION VS. ML ESTIMATES = .12556-00

REGRESSION:

$$Y = 0.4636 + 4.60468 + 0.414635X_1 - 0.00108611X_2 - 0.0102051X_1^2 - 0.000000200779X_2^2 + 0.0000490078X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean Residual for Model (33 Data Points) = 18.46%

* Indicates significance at 0.05 probability level.

APPENDIX 8E - ANALYSIS OF ROCK BASS SAC-FRY BIOASSAY DATA. AQUEOUS H₂S AT PH 7.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - TRANSFORMED DATA

SOURCE	SS	D.F.	MS	ADDFRY. F	MLE
REGRESSION	51608.6736	5	10321.77	40.47	.466076-2*
LINEAR	32343.0901	2	16171.55	54.74	.257040-1*
X1 LINEAR	8468.6756	1	8468.68	20.32	.425017-2*
X2 LINEAR	11156.0151	1	11156.02	142.57	.600064-2*
QUADRATIC	18062.0820	2	9031.04	52.91	.464557-1*
X1 QUAD.	6.6208	1	6.62	.04	.077306-0
X2 QUAD.	14084.5454	1	14084.55	90.97	.195707-1*
X1 * X2	3094.6136	1	3094.61	18.13	.110041-0*
TRANSFORM	13117.1972	1	13117.20	26.00	.270057-1*
RESIDUAL	10582.5571	62	170.69		
TOTAL	62191.6797	70			

RELATIVE LIKELIHOOD OF NO TRANSFORMATION VS. ML ESTIMATE = .000000-1

REGRESSION:

$$Y^{0.6233} = 17.8442 - 0.0000628931X_1^{2.556} - 0.0000136520X_2^{2.111} + (0.410714)10^{-7}X_1^{5.112} + (0.138582)10^{-10}X_2^{4.222} - (0.111041)10^{-7}X_1^{2.556}X_2^{2.111}$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean Residual for Model (71 Data Points) = 9.68%

* Indicates significance at 0.05 probability level

APPENDIX 8F - ANALYSIS OF ROCK BASS SAC-FRY BIOASSAY DATA.
 AQUEOUS H₂S AT PH 8.0 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - TRANSFORMED DATA

SOURCE	SS	D.F.	MS	APPROX. F	PUR
REGRESSION	3061.7251	1	4567.04	11.58	.126307-00 *
TEMPER	15547.0012	1	7773.50	19.73	.120510-00 *
% LINEAR	42.2006	1	42.20	.11	.036413-00
% LINEAR	14571.1633	1	14571.16	38.01	.107001-00 *
QUADRATIC	5621.1766	1	5621.18	7.13	.261500-00 *
% QUAD.	603.0722	1	603.07	1.06	.061364-00
% QUAD.	2675.2118	1	2675.21	13.69	.003770-00 *
X ₁ * X ₂	2607.7062	1	2607.71	6.76	.003181-01 *
TRANSFORM	856.2926	1	856.29	2.24	.221100-00
RESIDUAL	13789.6509	42	328.32		
TOTAL	36609.3740	43			

RELATIVE LIFESPAN OF NO TRANSFORMATION VS. % ESTIMATES = .271111-00

REGRESSION:

$$Y = 0.769 - 1.08669 + 2.78821X_1 + 0.137224X_2 - 0.0509123X_1^2 - 0.000211771X_2^2 - 0.00555351X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean Residual for Model (42 Data Points) = 15.16%

* Indicates significance at 0.05 probability level.

APPENDIX 8G - ANALYSIS OF ROCK BASS SAC-FRY BIOASSAY DATA
 AQUEOUS H₂S AT PH 8.5 FOR 1 HOUR

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	29916.8301	5	5983.37	12.74 *
LINEAR	28230.0239	2	14115.01	30.05 *
X1 LINEAR	3503.4635	1	3503.46	7.46 *
X2 LINEAR	15457.5109	1	15457.51	32.91 *
QUADRATIC	2793.8978	2	1396.95	2.97
X1 QUAD.	2628.6815	1	2628.68	5.60 *
X2 QUAD.	583.8203	1	583.52	1.22
X1 * X2	17.0988	1	7.10	
RESIDUAL	12682.6240	27	469.73	
TOTAL	42599.4541	32		

REGRESSION:

$$Y = -94.4611 + 18.3502X_1 - 0.291164X_2 - 0.405465X_1^2 + 0.000167072X_2^2 + 0.00112667X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean Residual for Model (33 Data Points) = 16.33%

* Indicates significance at 0.05 probability level.

APPENDIX 9A
RAINBOW TROUT SAC-FRY
96 HOUR BIOASSAY AT pH 7.5

Design H ₂ S Level	Design Temp °C	Observed Temp * °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	% Survival
0 (Control)	4.0	5.48 ± 0.29	7.61 ± 0.05	0	0	100 100 100
	12.0	12.01 ± 0.19	7.59 ± 0.01	0	0	100 100 100
	20.0	19.82 ± 0.27	7.69 ± 0.03	0	0	100 100 100
1	8.0	8.46 ± 0.14 8.23 ± 0.14 8.23 ± 0.12	7.51 ± 0.03 7.52 ± 0.03 7.52 ± 0.03	38.21 ± 3.86 30.02 ± 4.25 34.18 ± 3.37	12.91 ± 0.99 10.08 ± 1.21 11.52 ± 1.16	100 90 100
	16.0	16.31 ± 0.43 16.26 ± 0.42 16.23 ± 0.41	7.51 ± 0.02 7.56 ± 0.03 7.53 ± 0.03	32.54 ± 5.45 31.57 ± 6.61 27.19 ± 6.43	9.22 ± 1.34 8.31 ± 2.03 7.48 ± 1.84	100 100 100
2	4.0	4.95 ± 0.13 4.85 ± 0.17 5.26 ± 0.83	7.56 ± 0.06 7.57 ± 0.05 7.59 ± 0.05	87.86 ± 28.30 68.81 ± 12.74 76.90 ± 27.55	30.13 ± 7.66 23.14 ± 3.23 24.88 ± 6.62	90 90 100
	12.0	12.25 ± 0.27 12.91 ± 1.68 12.08 ± 0.19	7.52 ± 0.02 7.53 ± 0.02 7.56 ± 0.03	85.25 ± 9.10 97.73 ± 71.69 75.46 ± 10.60	25.97 ± 3.01 28.30 ± 19.07 21.75 ± 2.36	30 0 60
	20.0	19.23 ± 0.49 19.89 ± 0.34 19.83 ± 0.32	7.66 ± 0.06 7.67 ± 0.03 7.67 ± 0.02	92.77 ± 59.65 56.80 ± 11.21 58.86 ± 17.20	18.73 ± 11.07 11.51 ± 2.14 11.88 ± 3.46	0 70 60
3	8.0	8.35 ± 0.13 8.39 ± 0.13 8.40 ± 0.12	7.53 ± 0.02 7.52 ± 0.03 7.54 ± 0.04	123.09 ± 12.54 116.50 ± 15.19 113.62 ± 11.54	40.41 ± 4.31 39.27 ± 6.27 36.93 ± 4.92	10 0 0
	16.0	16.44 ± 0.61 16.54 ± 0.90 16.34 ± 0.42	7.51 ± 0.03 7.56 ± 0.04 7.53 ± 0.04	116.89 ± 24.03 162.32 ± 51.55 102.91 ± 16.65	33.32 ± 7.69 42.72 ± 15.88 28.12 ± 4.10	0 0 20
4	12.0	12.28 ± 0.18 12.40 ± 0.15 12.28 ± 0.18	7.57 ± 0.02 7.55 ± 0.02 7.56 ± 0.04	168.50 ± 48.14 173.89 ± 58.65 179.39 ± 67.80	47.79 ± 13.92 50.27 ± 17.26 51.09 ± 19.75	0 0 0

* Observed temperature, pH and H₂S values are time-weighted means
±95% confidence intervals for observations at 0800 and 1400
hours each day of the test.

APPENDIX 9A CONTINUED
RAINBOW TROUT SAC-FRY
96 HOUR BIOASSAY AT pH 8.0

Design H ₂ S Level	Design Temp °C	Observed Temp * °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	% Survival
0 (Control)	4.0	4.53 ± 0.11	8.02 ± 0.08	0	0	100 100 100
	12.0	11.89 ± 0.20	8.08 ± 0.03	0	0	100 100 100
	20.0	19.33 ± 0.35	8.17 ± 0.04	0	0	100 100 100
1	8.0	8.31 ± 0.06 8.27 ± 0.05 8.18 ± 0.10	8.01 ± 0.06 8.01 ± 0.06 8.01 ± 0.07	66.30 ± 6.90 60.85 ± 6.11 59.15 ± 7.01	9.26 ± 1.62 8.59 ± 1.44 8.32 ± 1.64	100 100 100
	16.0	15.48 ± 0.24 15.44 ± 0.27 15.48 ± 0.26	8.09 ± 0.04 8.09 ± 0.04 8.08 ± 0.03	50.26 ± 3.18 49.94 ± 4.27 43.63 ± 4.82	4.96 ± 0.53 4.84 ± 0.42 4.32 ± 0.54	100 100 100
	20.0	19.33 ± 0.35	8.17 ± 0.04	0	0	100 100 100
2	4.0	4.57 ± 0.14 4.52 ± 0.12 4.54 ± 0.10	8.01 ± 0.08 8.01 ± 0.08 8.00 ± 0.08	117.93 ± 7.77 99.04 ± 5.00 126.77 ± 22.21	18.70 ± 2.85 15.92 ± 2.54 20.00 ± 5.69	100 100 100
	12.0	12.08 ± 0.19 12.14 ± 0.17 11.91 ± 0.21	8.05 ± 0.05 8.05 ± 0.05 8.05 ± 0.04	135.12 ± 7.89 105.87 ± 8.74 123.46 ± 17.01	15.66 ± 1.49 12.16 ± 1.30 14.41 ± 2.73	60 80 70
	20.0	19.28 ± 0.29 19.28 ± 0.33 19.30 ± 0.30	8.14 ± 0.04 8.15 ± 0.04 8.14 ± 0.03	131.10 ± 13.68 77.76 ± 7.83 108.16 ± 12.74	10.42 ± 1.22 6.08 ± 0.60 8.54 ± 0.67	70 100 90
3	8.0	8.26 ± 0.09 8.29 ± 0.05 8.36 ± 0.07	8.01 ± 0.05 8.01 ± 0.05 8.00 ± 0.07	194.28 ± 20.51 193.78 ± 19.97 225.14 ± 17.59	27.44 ± 3.65 27.36 ± 4.20 32.05 ± 5.33	20 30 0
	16.0	15.39 ± 0.31 15.46 ± 0.25 15.53 ± 0.25	8.07 ± 0.05 8.07 ± 0.04 8.07 ± 0.03	176.98 ± 19.94 159.60 ± 25.24 182.53 ± 12.53	18.04 ± 2.14 16.18 ± 3.90 18.73 ± 1.78	0 40 0
	20.0	19.33 ± 0.35	8.17 ± 0.04	0	0	100 100 100
4	12.0	12.13 ± 0.57 12.25 ± 0.44 12.23 ± 0.28	8.04 ± 0.08 8.03 ± 0.07 8.04 ± 0.05	278.06 ± 48.96 277.90 ± 43.39 304.63 ± 45.06	33.04 ± 9.27 33.30 ± 8.68 35.99 ± 7.27	0 0 0

*Observed temperature, pH and H₂S values are time-weighted means
-95% confidence intervals for observations at 0800 and 1400 hours
each day of the test.

APPENDIX 9A CONTINUED
RAINBOW TROUT SAC-FRY
96 HOUR BIOASSAY AT pH 8.5

Design H ₂ S Level	Design Temp °C	Observed Temp °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	% Survival
0 (Control)	4.0	5.63±0.15	8.54±.08	0	0	100
		5.63±0.15	8.54±.08	0	0	100
		5.63±0.15	8.54±.08	0	0	100
	12.0	11.68±0.15	8.60±.07	0	0	100
		11.68±0.15	8.60±.07	0	0	100
		11.68±0.15	8.60±.07	0	0	100
	20.0	19.82±0.39	8.57±.04	0	0	100
		19.82±0.39	8.57±.04	0	0	100
		19.82±0.39	8.57±.04	0	0	100
1	8.0	8.04±0.16	8.56±.07	163.26± 15.67	7.41±1.75	100
		8.02±0.14	8.57±.07	163.26± 18.16	7.35±1.73	100
		7.96±0.14	8.57±.07	157.99± 13.77	7.07±1.46	100
	16.0	15.84±0.10	8.63±.04	132.79± 14.37	4.02±0.78	100
		15.74±0.08	8.62±.04	103.08± 31.57	3.19±1.13	100
		15.75±0.10	8.62±.05	115.25± 17.99	3.59±0.92	100
2	4.0	4.59±0.05	8.51±.08	345.69± 27.98	19.92±4.93	80
		4.53±0.07	8.51±.08	320.16± 25.81	18.56±4.76	100
		4.64±0.10	8.51±.09	346.06± 24.31	19.94±4.86	90
	12.0	11.79±0.16	8.58±.08	332.76± 35.35	13.13±3.52	30
		11.90±0.23	8.58±.07	300.01± 31.10	11.78±3.01	20
		11.74±0.17	8.59±.07	301.04± 34.50	11.64±3.22	20
	20.0	19.51±1.03	8.54±.08	487.49±300.51	16.39±12.03	0
		19.82±0.39	8.56±.04	220.83± 32.80	7.10± 1.49	50
		19.82±0.39	8.56±.03	304.95± 56.62	9.96± 2.38	50
3	8.0	8.09±0.15	8.55±.08	506.82± 32.14	23.31± 4.48	0
		8.06±0.20	8.52±.08	486.04± 37.94	23.87± 3.56	0
		8.06±0.30	8.45±.05	552.22± 36.08	30.98± 2.75	0
	16.0	15.73±0.07	8.60±.06	391.32± 16.74	12.63± 1.81	0
		15.76±0.17	8.57±.07	389.07± 23.81	13.44± 2.53	0
		15.78±0.15	8.56±.08	454.58± 35.47	16.14± 3.33	0
4	12.0	12.80±2.13	8.46±.07	692.77± 44.93	33.06± 5.30	0
		11.93±0.25	8.49±.07	628.58± 28.81	28.76± 5.37	0
		11.84±0.20	8.47±.07	706.89± 45.19	33.76± 5.76	0

APPENDIX 9B - ANALYSIS OF RAINBOW TROUT SAC-FRY BIOASSAY DATA.
TOTAL H₂S AT PH 7.5 FOR 96 HOURS

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
Regression	53345.2109	5	10669.04	26.66*
Linear	41869.8647	2	20934.93	52.31*
X1 Linear	2165.7550	1	2165.76	5.41*
X2 Linear	39719.1611	1	39719.16	99.24*
Quadratic	588.2075	2	294.10	.73
X1 Quad.	451.1615	1	451.16	1.13
1x Quad.	231.1767	1	231.18	.58
X1 X2	699.9551	1	699.96	1.75
Residual	10806.2983	27		
TOTAL	64151.5093	32		

REGRESSION:

$$Y = 135.899 - 5.02482X_1 - 0.538617X_2 + 0.185821X_1^2 + 0.000888260X_2^2 - 0.0213020X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean residual for Model (33 Data Points) = 15.36%

* Indicates significance at 0.05 probability level.

APPENDIX 9C - ANALYSIS OF RAINBOW TROUT SAC-FRY BIOASSAY DATA.
TOTAL H₂S AT pH 8.0 FOR 96 HOURS

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	C.F.	MSS	F
REGRESSION	44140.5801	5	8828.12	30.23 *
LINEAR	32460.8096	2	16230.40	55.59 *
X1 LINEAR	644.6202	1	644.62	2.21
X2 LINEAR	32384.4321	1	32384.43	110.91 *
QUADRATIC	1898.0879	2	949.04	3.25
X1 QUAD.	735.2611	1	735.26	2.52
X2 QUAD.	840.0266	1	840.03	2.88
X1 * X2	198.6780	1	198.68	.68
RESIDUAL	7883.6587	27	291.99	
TOTAL	52024.2388	32		

REGRESSION:

$$Y = 136.727 - 5.93821X_1 - 0.125884X_2 + 0.236626X_1^2 - 0.000617117X_2^2 - 0.00797916X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean residual for Model (33 Data Points) = 12.75%

* Indicates significance at 0.05 probability level.

APPENDIX 9D - ANALYSIS OF RAINBOW TROUT SAC-FRY BIOASSAY DATA.
TOTAL H₂S AT PH 8.5 FOR 96 HOURS

ANALYSIS OF VARIANCE TABLE - TRANSFORMED DATA

SOURCE	SS	D.F.	MSS	APPROX. F	MLR
REGRESSION	3721.7083	5	744.34	51.70	.214E-17-17 *
LINEAR	2603.6926	2	1301.85	90.43	.431E-15 *
X1 LINEAR	239.1016	1	239.10	16.61	.170E-03 *
X2 LINEAR	2216.3472	1	2216.35	153.95	.440E-14 *
QUADRATIC	612.2018	2	306.10	21.26	.494E-07 *
X1 QUAD.	97.1111	1	97.11	6.75	.167E-01 *
X2 QUAD.	575.7658	1	575.77	39.99	.938E-07 *
X1 * X2	49.0156	1	49.02	3.40	.112E+00
TRANSFORM	10316.1526	3	3438.72	238.80	.266E+08-24 *
RESIDUAL	345.5243	24	14.40		
TOTAL	4067.2326	32			

RELATIVE LIKELIHOOD OF NO TRANSFORMATION VS. ML ESTIMATES = .266E-24

REGRESSION:

$$Y^{0.3269} = 298.924 - 491.166X_1 + 0.000521464X_2 + 204.810X_1^{0.148} + (0.360983)10^{-10}X_2^{4.00} - 0.0000655612X_1^{0.074}X_2^{2.00}$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean residual for Model (33 Data Points) = 16.97%

* Indicates significance at 0.05 probability level.

APPENDIX 9E - ANALYSIS OF RAINBOW TROUT SAC-FRY BIOASSAY DATA.
AQUEOUS H₂S AT PH 7.5 FOR 96 HOURS

ANALYSIS OF VARIANCE TABLE - TRANSFORMED DATA

SOURCE	SS	D.F.	MSS	APPROX. F	MLR
REGRESSION	5351.8042	5	1070.36	26.32	.403243-13 *
LINEAR	2539.2692	2	1269.63	31.22	.657474-09 *
X1 LINEAR	691.2910	1	691.29	17.00	.145509-03 *
X2 LINEAR	1896.4300	1	1896.43	46.63	.184101-07 *
QUADRATIC	2819.0514	2	1409.53	34.66	.185828-09 *
X1 QUAD.	31.3115	1	31.31	.77	.593924-00
X2 QUAD.	2536.0052	1	2536.01	62.36	.667629-09 *
X1 * X2	318.0585	1	318.06	7.84	.942643-02 *
TRANSFORM	9889.8040	1	9889.80	81.06	.538575-17 *
RESIDUAL	976.0516	24	40.67		
TOTAL	6327.8558	32			

RELATIVE LIKELIHOOD OF NO TRANSFORMATION VS. ML ESTIMATES = .53857-17

REGRESSION:

$$Y = 108.884 - 258.796X_1 + 137.051X_2 + 119.141X_1 - 49.3592X_2 - 40.6163X_1 X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean Residual for Model (33 Data Points) = 11.92%

* Indicates significance at 0.05 probability level.

APPENDIX 9F - ANALYSIS OF RAINBOW TROUT SAC-FRY BIOASSAY DATA.
AQUEOUS H₂S AT pH 8.0 FOR 96 HOURS

ANALYSIS OF VARIANCE TABLE - TRANSFORMED DATA

SOURCE	SS	D.F.	MSS	APPROX. F	MLR
REGRESSION	18851.4207	5	3770.28	35.07	.676073-15 *
LINEAR	14896.5125	2	7448.26	69.28	.195800-13 *
X1 LINEAR	3084.0017	1	3084.00	28.69	.232042-05 *
X2 LINEAR	13656.3083	1	13656.31	127.02	.659580-13 *
QUADRATIC	1947.5802	2	973.79	9.06	.933752-04 *
X1 QUAD.	8.9445	1	8.94	.08	.944501+00
X2 QUAD.	1910.7365	1	1910.74	17.77	.106851-03 *
X1 * X2	1508.6984	1	1508.70	14.03	.502143-03 *
TRANSFORM	4351.7515	3	1450.58	13.48	.828421-07 *
RESIDUAL	2580.2495	24	107.51		
TOTAL	21431.6702	32			

RELATIVE LIKELIHOOD OF NO TRANSFORMATION VS. PL ESTIMATES = .82842-07

REGRESSION:

$$Y^{0.5725} = 141.193 - 202.455X_1^{0.074} + 0.103356X_2^{2.222} + 80.6763X_1^{0.148} + 0.00000339614X_2^{4.444} - 0.0982478X_1^{0.074} X_2^{2.222}$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean residual for model (33 Data Points) = 9.53%

* Indicates significance at 0.05 probability level.

APPENDIX 9G - ANALYSIS OF RAINBOW TROUT SAC-FRY BIOASSAY DATA.
AQUEOUS H₂S AT PH 8.5 FOR 96 HOURS

ANALYSIS OF VARIANCE TABLE - TRANSFORMED DATA

SOURCE	SS	C.F.	MSS	APPROX. F	MLR
REGRESSION.					
LINEAR	3249.1341	5	649.83	26.17	.436203-13*
X1 LINEAR	2530.3135	2	1265.16	50.95	.132656-11*
X2 LINEAR	704.0509	1	704.05	28.35	.257444-05*
GLADRATIC	2269.6627	1	2269.66	92.21	.497424-11*
X1 QUAD.	490.8079	2	245.40	9.88	.494989-04*
X2 QUAD.	471.6553	1	471.66	1.91	.282275+00
X1 * X2	215.9701	1	215.97	19.00	.663765-04*
TRANSFORM	10980.9163	3	3660.31	8.70	.607580-02*
RESIDUAL	595.9178	24	24.83	147.46	.551243-21*
TOTAL	3845.0519	32			

RELATIVE LIKELIHOOD CF NO TRANSFORMATION VS. ML ESTIMATES = .55124-21

REGRESSION:

$$Y^{0.289} = 195.007 - 317.063X_1^{0.074} + 0.213862X_2^{1.556} + 131.440X_1^{0.148} + 0.000127265X_2^{3.112} - 0.217029X_1^{0.074} X_2^{1.556}$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean residual for model (33 Data Points) = 21.51%

* Indicates significance at 0.05 probability level.

**APPENDIX 10A
RAINBOW TROUT JUVENILES
96 HOUR BIOASSAY AT pH 7.5**

Design H ₂ S Level	Design Temp °C	Observed Temp* °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	% Survival
0 (Control)	4.0	6.94±0.37	7.42±0.07	0	0	100
		6.94±0.37	7.42±0.07	0	0	100
		6.94±0.37	7.42±0.07	0	0	100
	12.0	12.33±0.09	7.39±0.08	0	0	100
		12.33±0.09	7.39±0.08	0	0	100
		12.33±0.09	7.39±0.08	0	0	100
	20.0	19.51±0.67	7.38±0.05	0	0	100
		19.51±0.67	7.38±0.05	0	0	100
		19.51±0.67	7.38±0.05	0	0	100
	4.0	6.88±0.46	7.54±0.15	0	0	100
		6.88±0.46	7.54±0.15	0	0	100
		6.88±0.46	7.54±0.15	0	0	100
	12.0	12.76±0.30	7.47±0.16	0	0	80
		12.76±0.30	7.47±0.16	0	0	100
		12.76±0.30	7.47±0.16	0	0	100
	20.0	20.02±0.16	7.34±0.17	0	0	80
		20.02±0.16	7.34±0.17	0	0	100
		20.02±0.16	7.34±0.17	0	0	100
1	8.0	8.99±0.21	7.34±0.07	44.26± 14.19	18.56± 5.97	100
		8.67±0.24	7.43±0.07	39.09± 11.24	14.51± 4.03	80
		8.69±0.15	7.38±0.06	49.04± 15.95	19.55± 6.36	100
	16.0	17.06±0.14	7.40±0.04	30.36± 7.75	10.00± 2.31	100
		17.02±0.16	7.52±0.05	32.52± 9.64	8.79± 2.42	100
		17.18±0.18	7.47±0.06	39.84± 9.88	11.68± 2.51	100
2	4.0	6.68±0.25	7.51±0.07	97.16± 28.16	33.59± 9.29	100
		6.58±1.26	7.57±0.10	97.73± 23.43	31.59± 8.73	100
		5.98±0.48	7.60±0.08	93.08± 18.31	29.02± 6.56	100
		6.04±0.43	7.67±0.14	140.22± 27.30	41.02± 17.19	100
		5.47±0.35	7.69±0.15	138.22± 39.60	39.56± 20.95	80
		5.44±0.26	7.68±0.14	150.35± 25.87	43.65± 16.90	100
	12.0	12.51±0.07	7.48±0.06	83.54± 21.65	26.64± 6.15	100
		12.56±0.13	7.49±0.05	81.74± 20.34	25.48± 5.74	80
		12.23±0.10	7.43±0.06	97.93± 23.34	33.97± 7.67	80
		13.33±0.26	7.53±0.17	107.60± 29.28	31.80± 16.05	100
		13.43±0.22	7.53±0.17	84.44± 18.18	25.47± 11.75	80
		13.36±0.31	7.53±0.18	117.91± 31.83	36.13± 18.85	40
	20.0	19.87±0.40	7.45±0.04	65.53± 16.89	19.16± 4.39	100
		20.01±0.32	7.49±0.05	76.66± 18.19	20.81± 4.26	60
		19.78±0.33	7.50±0.06	55.39± 14.39	14.70± 2.78	80
		20.05±0.15	7.49±0.15	112.12± 44.40	32.74± 19.99	40
		19.97±0.18	7.55±0.15	75.59± 21.07	19.18± 7.64	100
		19.82±0.10	7.54±0.16	59.44± 24.87	14.82± 8.18	60
3	8.0	8.64±0.29	7.53±0.20	222.08± 35.21	73.47± 26.22	40
		8.20±0.32	7.52±0.24	250.43± 42.04	85.04± 36.25	0
		9.02±0.43	7.54±0.19	214.62± 46.66	69.09± 33.64	40
	16.0	17.07±0.61	7.44±0.18	201.81± 29.28	64.78± 20.49	20
		16.23±0.25	7.37±0.74	320.13± 309.38	104.10± 100.51	0
		17.13±0.59	7.47±0.20	139.25± 64.40	55.04± 26.24	20
4	12.0	13.31±0.52	7.34±0.71	384.84± 369.78	138.01± 127.44	0
		13.35±1.25	7.38±0.76	358.63± 266.54	125.40± 120.34	0
		13.24±0.75	7.38±0.79	333.00± 317.00	113.42± 126.92	0

*Observed temperature, pH and H₂S values are time-weighted means ±95% confidence intervals for observations at 0800 and 1400 hrs each day of the test.

APPENDIX 10A CONTINUED
RAINBOW TROUT JUVENILES
96 HOUR BIOASSAY AT pH 8.0

Design H ₂ S Level	Design Temp °C	Observed Temp * °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	% Survival
0 (Control)	4.0	7.35 ± 0.29	7.67 ± 0.24	0	0	100 100 100
	12.0	12.40 ± 0.32	7.81 ± 0.22	0	0	100 100 100
	20.0	19.87 ± 0.26	7.68 ± 0.19	0	0	100 100 100
1	8.0	8.92 ± 0.31 8.43 ± 0.29 8.65 ± 0.19	7.84 ± 0.12 7.90 ± 0.08 7.87 ± 0.08	108.13 ± 14.79 89.92 ± 13.91 111.42 ± 16.70	21.16 ± 5.64 15.74 ± 3.58 20.55 ± 4.60	100 100 80
	16.0	15.94 ± 0.45 15.93 ± 0.51 15.91 ± 0.51	7.96 ± 0.06 8.01 ± 0.07 7.98 ± 0.06	64.58 ± 9.92 86.44 ± 12.73 96.81 ± 16.73	8.10 ± 1.61 9.80 ± 1.83 11.72 ± 2.60	100 80 80
2	4.0	6.37 ± 0.88 6.50 ± 0.31 6.18 ± 0.50	7.83 ± 0.08 7.83 ± 0.06 7.82 ± 0.07	211.25 ± 51.08 213.33 ± 36.01 196.85 ± 34.75	44.34 ± 14.19 44.62 ± 9.56 42.83 ± 10.23	60 80 100
	12.0	12.86 ± 0.26 12.75 ± 0.26 12.39 ± 0.24	8.00 ± 0.06 7.94 ± 0.08 7.94 ± 0.07	194.04 ± 40.95 181.90 ± 38.52 239.60 ± 52.89	25.20 ± 8.18 26.49 ± 8.39 34.90 ± 10.77	100 80 100
	20.0	19.88 ± 0.22 19.88 ± 0.16 19.97 ± 0.13	7.94 ± 0.10 7.90 ± 0.08 7.88 ± 0.08	182.54 ± 42.03 176.00 ± 36.25 134.81 ± 29.16	23.31 ± 10.24 23.32 ± 6.92 18.05 ± 5.55	40 100 80
3	8.0	8.44 ± 0.26 8.06 ± 0.41 9.23 ± 0.31	7.98 ± 0.04 7.98 ± 0.05 7.86 ± 0.08	335.31 ± 62.05 368.17 ± 83.28 332.63 ± 69.09	49.32 ± 9.66 54.91 ± 14.47 60.41 ± 16.27	60 20 20
	16.0	15.73 ± 0.51 15.85 ± 0.48 16.12 ± 0.54	8.03 ± 0.07 8.00 ± 0.05 7.98 ± 0.09	271.56 ± 53.57 326.48 ± 55.60 300.29 ± 71.10	30.37 ± 8.13 37.86 ± 8.02 37.07 ± 12.58	20 0 40
4	12.0	12.23 ± 0.25 12.26 ± 0.26 12.46 ± 0.53	7.95 ± 0.05 7.97 ± 0.04 7.97 ± 0.08	494.92 ± 118.67 513.00 ± 107.20 538.36 ± 227.99	70.42 ± 16.67 69.42 ± 15.38 71.14 ± 27.77	0 0 0

* Observed temperature, pH and H₂S values are time-weighted means
±95% confidence intervals for observations at 0800 and 1400
hours each day of the test.

RAINBOW TROUT JUVENILES
96 HOUR BIOASSAY AT pH 8.5

Design H ₂ S Level	Design Temp °C	Observed Temp * °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	% Survival
0 (Control)	4.0	7.28 ± 0.58	8.00 ± 0.25	0	0	100 100 100
	12.0	11.69 ± 0.34	8.39 ± 0.16	0	0	100 100 100
	20.0	19.47 ± 0.35	8.17 ± 0.30	0	0	100 100 100
1	8.0	8.10 ± 0.66	8.54 ± 0.13	182.97 ± 19.89	8.98 ± 2.32	100
		8.19 ± 0.74	8.46 ± 0.15	128.69 ± 26.92	7.31 ± 2.26	100
		8.06 ± 0.52	8.48 ± 0.16	193.03 ± 24.61	10.93 ± 3.14	100
	16.0	15.58 ± 0.48	8.53 ± 0.10	115.91 ± 21.84	4.51 ± 1.25	80
		15.73 ± 0.68	8.42 ± 0.14	161.97 ± 24.88	8.25 ± 2.49	20
		15.79 ± 0.59	8.43 ± 0.13	197.16 ± 28.52	9.54 ± 2.50	100
2	4.0	6.91 ± 1.38	8.26 ± 0.24	369.84 ± 88.32	33.57 ± 9.05	80
		6.53 ± 1.05	8.28 ± 0.25	389.91 ± 80.71	35.20 ± 9.93	80
		6.16 ± 0.72	8.29 ± 0.24	346.03 ± 81.55	32.28 ± 9.83	80
	12.0	12.05 ± 0.30	8.54 ± 0.13	323.50 ± 40.93	13.89 ± 3.65	60
		12.05 ± 0.24	8.54 ± 0.12	328.59 ± 47.49	14.00 ± 3.71	80
		11.64 ± 0.36	8.51 ± 0.13	430.53 ± 150.76	20.06 ± 9.25	20
	20.0	19.47 ± 0.58	8.28 ± 0.13	363.78 ± 54.42	21.43 ± 4.41	40
		19.60 ± 0.43	8.27 ± 0.13	344.63 ± 56.69	21.16 ± 5.71	40
		19.55 ± 0.61	8.28 ± 0.14	291.75 ± 101.26	17.59 ± 7.03	60
3	8.0	8.16 ± 0.41	8.48 ± 0.14	561.16 ± 88.19	30.36 ± 7.25	60
		7.82 ± 0.45	8.45 ± 0.12	629.69 ± 113.25	36.33 ± 8.69	40
		8.63 ± 0.47	8.43 ± 0.16	640.72 ± 96.48	37.72 ± 9.17	40
	16.0	15.37 ± 0.58	8.47 ± 0.12	556.66 ± 66.91	24.48 ± 5.86	100
		15.80 ± 0.57	8.44 ± 0.12	658.56 ± 89.36	30.81 ± 7.43	40
		16.19 ± 0.88	8.34 ± 0.15	541.81 ± 105.70	31.22 ± 9.77	20
4	12.0	11.66 ± 0.34	8.47 ± 0.09	774.38 ± 125.84	37.85 ± 8.04	0
		11.68 ± 0.43	8.46 ± 0.10	859.22 ± 116.72	42.98 ± 9.96	20
		11.65 ± 0.31	8.48 ± 0.09	743.75 ± 92.57	35.61 ± 7.46	20

* Observed temperature, pH and H₂S values are time-weighted means
±95% confidence intervals for observations at 0800 and 1400
hours each day of the test.

APPENDIX 10B - ANALYSIS OF RAINBOW TROUT JUVENILE BIOASSAY DATA.
TOTAL H₂S AT PH 7.5 FOR 96 HOURS

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA			
SOURCE	SS	D.F.	F
REGRESSION	46658.1855	5	36.64 *
LINEAR	41056.3350	2	80.61 *
X1 LINEAR	2962.3128	1	11.63 *
X2 LINEAR	40025.7051	1	157.18 *
QUADRATIC	171.1305	2	.34
X1 QUAD.	124.2997	1	.49
X2 QUAD.	23.8091	1	.09
X1 * X2	718.8805	1	2.82
RESIDUAL	11459.4556	45	
TOTAL	58117.6411	50	

REGRESSION:

$$Y = 122.291 - 2.87951X_1 - 0.119694X_2 + 0.0857767X_1^2 - 0.0000616637X_2 - 0.0116309X_1X_2$$

Y = % Survival

X = Temperature °C

X = Total H₂S ppb

Mean Residual for Model (51 Data Points) = 11.18%

* Indicates significance at 0.05 probability level.

APPENDIX 10C - ANALYSIS OF RAINBOW TROUT JUVENILE BIOASSAY DATA.
TOTAL H₂S AT PH 8.0 FOR 96 HOURS

ANALYSIS OF VARIANCE TABLE - TRANSFORMED DATA

SOURCE	SS	D.F.	MSS	APPROX. F	MLR
REGRESSION	23981.8435	5	4796.37	30.89	.130709-13 *
LINEAR	18869.0640	2	9434.53	60.77	.363143-12 *
X ₁ LINEAR	814.1302	1	814.13	5.24	.482410-01 *
X ₂ LINEAR	18865.9780	1	18865.98	121.52	.363951-12 *
QUADRATIC	2247.3373	2	1123.67	7.24	.673566-03 *
X ₁ QUAD.	2.7052	1	2.71	.02	.989006+00
X ₂ QUAD.	2172.5700	1	2172.57	13.99	.820641-03 *
X ₁ * X ₂	551.2922	1	551.29	3.55	.120559+00
TRANSFORM	5069.4231	1	5069.42	32.65	.147096-05 *
RESIDUAL	4036.8154	26	155.25		
TOTAL	28918.3589	32			

RELATIVE LIKELIHOOD OF NO TRANSFORMATION VS. ML ESTIMATES = .14800-05

REGRESSION:

$$Y^{0.4492} = 7.46327 + 0.067156X_1 + 0.00767686X_2 - 0.00159297X_1^2 - 0.0000278138X_2^2 - 0.000769932X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean Residual for Model (33 Data Points) = 11.39%

* Indicates significance at 0.05 probability level.

APPENDIX 10D - ANALYSIS OF RAINBOW TROUT JUVENILE BIOASSAY DATA
TOTAL H₂S AT PH 8.5 FOR 96 HOURS

ANALYSIS OF VARIANCE TABLE - TRANSFORMED DATA

SOURCE	SS	D.F.	MSS	APPROX. F	MLR
REGRESSION	24310.0652	5	4862.01	14.89	.770531-10.*
LINEAR	15765.8039	2	7882.90	24.14	.126055-07.*
X ₁ LINEAR	1362.9200	1	1362.92	4.17	.710004-01.*
X ₂ LINEAR	14835.7374	1	14835.74	45.42	.244710-07.*
QUADRATIC	7622.3061	2	3811.15	11.67	.135702-04.*
X ₁ QUAD.	21.4730	1	21.47	.07	.955866+00
X ₂ QUAD.	7064.0289	1	7064.03	21.63	.248057-04.*
X ₁ * X ₂	307.6619	1	307.66	1.22	.441968+00
TRANSFORM	489.3770	1	489.38	0.50	.368150+00
RESIDUAL	7838.6626	24			
TOTAL	32148.7278	30			

RELATIVE LIKELIHOOD OF NO TRANSFORMATION VS. ML ESTIMATES = .36816+00

REGRESSION:

$$Y = 936.921 - 1455.36X_1 + 72.8851X_2 + 603.556X_1 - 7.39985X_2$$

0.9063 0.074 0.222 0.148 0.444

- 41.8818X₁ 0.074 0.222 X₂

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean Residual for Model (33 Data Points) = 9.05%

* Indicates significance at 0.05 probability level.

APPENDIX 10E - ANALYSIS OF RAINBOW TROUT JUVENILE BIOASSAY DATA.
AQUEOUS H₂S AT PH 7.5 FOR 96 HOURS

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA				
SOURCE	SS	D.F.	MS	F
REGRESSION	47228.0569	5	9445.77	39.04 *
LINEAR	41601.8960	2	20800.95	85.96 *
X1 LINEAR	3311.2369	1	3311.24	13.68 *
X2 LINEAR	40359.5044	1	40359.50	166.79 *
QUADRATIC	108.2877	2	54.14	.22
X1 QUAD.	43.3683	1	43.37	.18
X2 QUAD.	78.1075	1	78.11	.32
X1 * X2	729.5143	1	729.51	3.01
RESIDUAL	10888.7842	45	241.97	
TOTAL	58117.6411	50		

REGRESSION:

$$Y = 119.374 - 2.07909X_1 - 0.486831X_2 + 0.0508387X_1^2 + 0.000924161X_2^2$$

$$- 0.0366593X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean residual for Model (51 Data Points) = 11.77%

* Indicates significance at 0.05 probability level.

APPENDIX 10F - ANALYSIS OF RAINBOW TROUT JUVENILE BIOASSAY DATA.
 AQUEOUS H₂S AT PH 8.0 FOR 96 HOURS

Analysis of Variance Table - Untransformed Data

Source	SS	D.F.	MSS	F
Regression	3387.8853	3	6777.53	17.97 *
Linear	27977.7295	2	13933.35	37.03 *
X1 Linear	3840.0027	1	3340.00	10.18 *
X2 Linear	27907.1737	1	27907.18	73.93 *
Quadratic	1183.0213	2	591.51	1.57
X1 Quad	21.0038	1	21.01	0.06
X2 Quad	1075.4230	1	1075.42	2.85
X1 . X2	1469.3316	1	1159.33	3.90
Residual	10184.8389	27		
TOTAL	44072.7241	32		

REGRESSION:

$$Y = 112.410 - 1.57856X_1 + 0.486747X_2 + 0.0510366X_1^2 - 0.0118586X_2^2 - 0.0979977X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean residual for model (33 Data Points) = 12.39%

* Indicates significance at 0.05 probability level.

APPENDIX 10G - ANALYSIS OF RAINBOW TROUT JUVENILE BIOASSAY DATA.
 AQUEOUS H₂S AT pH 8.5 FOR 96 HOURS

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	24900.5535	5	4980.11	16.87 *
LINEAR	22350.0520	2	11175.03	37.85 *
X1 LINEAR	3402.4871	1	3402.49	11.52 *
X2 LINEAR	22052.5640	1	22052.57	74.60 *
QUADRATIC	1012.6175	2	506.31	1.71
X1 QUAD.	512.8706	1	512.87	1.74
X2 QUAD.	390.5718	1	390.57	1.32
X1 * X2	1435.1390	1	1435.14	4.86 *
RESIDUAL	7972.1724	27	295.27	
TOTAL	32872.7256	32		

REGRESSION:

$$Y = 141.389 - 6.96288X_1 + 0.550158X_2 + 0.253658X_1^2 - 0.0250131X_2^2 - 0.126477X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean residual for model (33 Data Points) = 10.24%

* Indicates significance at 0.05 probability level.

APPENDIX 11A
LAKE WHITEFISH SAC-FRY
96 HOUR BIOASSAY AT pH 7.5

Design H ₂ S Level	Design Temp °C	Observed Temp * °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	% Survival
0 (Control)	4.0	4.90 ± 0.12	7.72 ± 0.10	0	0	100 100 100
	10.0	9.81 ± 4.6	7.62 ± 0.08	0	0	90 100 100
	16.0	16.35 ± 0.18	7.73 ± 0.09	0	0	90 50 100
1	7.0	6.78 ± 0.14	7.59 ± 0.07	3.78 ± 6.43	1.35 ± 2.51	100
		6.73 ± 0.23	7.59 ± 0.09	2.77 ± 3.44	0.97 ± 1.36	100
		6.60 ± 0.29	7.59 ± 0.08	2.61 ± 2.40	0.84 ± 0.83	90
	13.0	12.78 ± 0.42	7.62 ± 0.07	2.93 ± 1.23	0.76 ± 0.34	90
		12.70 ± 0.46	7.60 ± 0.04	3.43 ± 1.59	0.91 ± 0.41	100
		12.69 ± 0.43	7.58 ± 0.06	4.29 ± 1.95	1.16 ± 0.45	100
2	4.0	4.50 ± 0.17	7.71 ± 0.08	10.71 ± 7.48	2.85 ± 1.88	100
		4.49 ± 0.14	7.67 ± 0.10	8.37 ± 5.70	2.44 ± 1.60	100
		4.46 ± 0.15	7.69 ± 0.11	6.86 ± 5.40	1.92 ± 1.46	90
	10.0	9.79 ± 0.51	7.49 ± 0.06	11.31 ± 4.14	3.83 ± 1.45	90
		9.89 ± 0.54	7.54 ± 0.05	13.57 ± 5.94	4.29 ± 2.04	90
		9.75 ± 0.52	7.50 ± 0.04	9.44 ± 4.30	3.20 ± 1.55	100
	16.0	16.10 ± 0.23	7.70 ± 0.09	13.00 ± 3.19	2.71 ± 0.76	100
		16.08 ± 0.24	7.72 ± 0.10	10.01 ± 3.93	1.98 ± 0.70	100
		16.07 ± 0.22	7.73 ± 0.10	10.68 ± 3.02	2.05 ± 0.46	90
3	7.0	6.86 ± 0.24	7.58 ± 0.07	18.49 ± 13.03	6.20 ± 5.34	80
		6.71 ± 0.27	7.59 ± 0.07	21.57 ± 16.74	7.21 ± 6.87	90
		6.75 ± 0.15	7.60 ± 0.08	19.19 ± 17.16	6.17 ± 6.02	90
	13.0	12.65 ± 0.47	7.61 ± 0.07	13.09 ± 6.97	3.34 ± 1.92	0
		12.71 ± 0.46	7.54 ± 0.05	16.75 ± 3.01	4.97 ± 1.11	80
		12.82 ± 0.37	7.60 ± 0.07	16.44 ± 4.81	4.26 ± 0.90	90
4	10.0	10.04 ± 0.44	7.57 ± 0.07	22.25 ± 9.45	6.71 ± 2.97	80
		10.13 ± 0.52	7.57 ± 0.03	28.52 ± 8.72	8.36 ± 2.29	50
		10.07 ± 0.53	7.64 ± 0.02	22.64 ± 6.03	5.97 ± 1.36	60

* Observed Temperature, pH and H₂S values are time-weighted means
±95% confidence intervals for observations at 0800 and 1400 hours
each day of the test.

APPENDIX 11A CONTINUED
LAKE WHITEFISH SAC-FRY
96 HOUR BIOASSAY AT pH 7.5 (TEST 2)

Design H ₂ S Level	Design Temp °C	Observed Temp ° °C	Observed pH	Total Sulphide ppB	Aqueous H ₂ S ppB	Survival
0 (Control)	4.0	4.99 ± 0.24	7.78 ± 0.03	0	0	100 100 100
	10.0	10.24 ± 0.12	7.71 ± 0.03	0	0	90 100 90
	16.0	16.43 ± 0.09	7.86 ± 0.03	0	0	90 70 90
1	7.0	6.79 ± 0.19 6.79 ± 0.26 6.58 ± 0.21	7.56 ± 0.03 7.58 ± 0.04 7.57 ± 0.03	7.35 ± 1.27 4.86 ± 1.09 5.70 ± 1.01	2.41 ± 0.44 1.54 ± 0.39 1.85 ± 0.36	100 100 100
	13.0	12.91 ± 0.08 12.83 ± 0.22 12.82 ± 0.18	7.59 ± 0.04 7.61 ± 0.04 7.62 ± 0.06	6.21 ± 1.17 11.27 ± 2.33 8.97 ± 1.53	1.68 ± 0.35 2.95 ± 0.70 2.31 ± 0.43	60 90 70
2	4.0	4.41 ± 0.26 4.52 ± 0.22 4.60 ± 0.26	7.69 ± 0.05 7.70 ± 0.03 7.73 ± 0.03	20.59 ± 1.26 13.15 ± 3.54 11.42 ± 3.25	5.81 ± 0.68 3.64 ± 0.89 3.04 ± 0.91	100 100 100
	10.0	10.25 ± 0.17 10.36 ± 0.20 10.20 ± 0.15	7.59 ± 0.03 7.66 ± 0.04 7.63 ± 0.04	15.67 ± 6.28 14.74 ± 4.06 17.53 ± 2.59	4.50 ± 1.81 3.79 ± 1.22 4.68 ± 0.53	90 80 100
	16.0	16.18 ± 0.12 16.25 ± 0.06 16.20 ± 0.08	7.80 ± 0.03 7.82 ± 0.03 7.83 ± 0.03	23.13 ± 3.85 19.25 ± 1.87 22.03 ± 3.66	3.95 ± 0.68 3.15 ± 0.36 3.58 ± 0.61	20 20 30
3	7.0	6.71 ± 0.20 6.69 ± 0.23 6.72 ± 0.16	7.55 ± 0.04 7.56 ± 0.04 7.61 ± 0.04	36.33 ± 5.57 34.09 ± 3.50 31.56 ± 1.73	12.16 ± 2.38 11.15 ± 1.58 9.61 ± 0.75	70 90 90
	13.0	12.81 ± 0.09 12.85 ± 0.10 12.88 ± 0.20	7.60 ± 0.04 7.61 ± 0.04 7.60 ± 0.04	34.89 ± 2.23 32.56 ± 2.08 32.94 ± 3.49	9.29 ± 1.06 8.46 ± 0.54 8.74 ± 1.22	80 80 90
4	10.0	11.23 ± 1.45 10.63 ± 0.17 10.62 ± 0.16	7.68 ± 0.05 7.68 ± 0.06 7.77 ± 0.05	41.27 ± 12.36 51.57 ± 18.65 45.37 ± 6.26	10.24 ± 3.18 12.93 ± 4.73 9.36 ± 1.47	100 30 60

*Observed temperature, pH and H₂S values are time-weighted means ±95% confidence intervals for observations at 0800 and 1400 hours each day of the test.

APPENDIX 11A CONTINUED
LAKE WHITEFISH SAC-FRY
96 HOUR BIOASSAY AT pH 7.5 (TEST 2)

Design H ₂ S Level	Design Temp °C	Observed Temp * °C	Observed pH	Total Sulphide ppB	Aqueous H ₂ S ppB	Survival
0 (Control)	4.0	4.99 ± 0.24	7.78 ± 0.03	0	0	100 100 100
	10.0	10.24 ± 0.12	7.71 ± 0.03	0	0	90 100 90
	16.0	16.43 ± 0.09	7.86 ± 0.03	0	0	90 70 90
1	7.0	6.79 ± 0.19 6.79 ± 0.26 6.58 ± 0.21	7.56 ± 0.03 7.58 ± 0.04 7.57 ± 0.03	7.35 ± 1.27 4.86 ± 1.09 5.70 ± 1.01	2.41 ± 0.44 1.54 ± 0.39 1.85 ± 0.36	100 100 100
	13.0	12.91 ± 0.08 12.83 ± 0.22 12.82 ± 0.18	7.59 ± 0.04 7.61 ± 0.04 7.62 ± 0.06	6.21 ± 1.17 11.27 ± 2.33 8.97 ± 1.53	1.68 ± 0.35 2.95 ± 0.70 2.31 ± 0.43	60 90 70
2	4.0	4.41 ± 0.26 4.52 ± 0.22 4.60 ± 0.26	7.69 ± 0.05 7.70 ± 0.03 7.73 ± 0.03	20.59 ± 1.26 13.15 ± 3.54 11.42 ± 3.25	5.81 ± 0.68 3.64 ± 0.89 3.04 ± 0.91	100 100 100
	10.0	10.25 ± 0.17 10.36 ± 0.20 10.20 ± 0.15	7.59 ± 0.03 7.66 ± 0.04 7.63 ± 0.04	15.67 ± 6.28 14.74 ± 4.06 17.53 ± 2.59	4.50 ± 1.81 3.79 ± 1.22 4.68 ± 0.53	90 80 100
	16.0	16.18 ± 0.12 16.25 ± 0.06 16.20 ± 0.08	7.80 ± 0.03 7.82 ± 0.03 7.83 ± 0.03	23.13 ± 3.85 19.25 ± 1.87 22.03 ± 3.66	3.95 ± 0.68 3.15 ± 0.36 3.58 ± 0.61	20 20 30
3	7.0	6.71 ± 0.20 6.69 ± 0.23 6.72 ± 0.16	7.55 ± 0.04 7.56 ± 0.04 7.61 ± 0.04	36.33 ± 5.57 34.09 ± 3.50 31.56 ± 1.73	12.16 ± 2.38 11.15 ± 1.58 9.61 ± 0.75	70 90 90
	13.0	12.81 ± 0.09 12.85 ± 0.10 12.88 ± 0.20	7.60 ± 0.04 7.61 ± 0.04 7.60 ± 0.04	34.89 ± 2.23 32.56 ± 2.08 32.94 ± 3.49	9.29 ± 1.06 8.46 ± 0.54 8.74 ± 1.22	80 80 90
4	10.0	11.23 ± 1.45 10.63 ± 0.17 10.62 ± 0.16	7.68 ± 0.05 7.68 ± 0.06 7.77 ± 0.05	41.27 ± 12.36 51.57 ± 18.65 45.37 ± 6.26	10.24 ± 3.18 12.93 ± 4.73 9.36 ± 1.47	100 30 60

*Observed temperature, pH and H₂S values are time-weighted means ± 95% confidence intervals for observations at 0800 and 1400 hours each day of the test.

APPENDIX 11A CONTINUED
LAKE WHITEFISH SAC-FRY
96 HOUR BIOASSAY AT pH 7.5 (TEST 3)

Design H ₂ S Level	Design Temp °C	Observed Temp * °C	Observed pH	Total Sulphide ppB	Aqueous H ₂ S ppB	% Survival
0 (Control)	4.0	5.51 ± 0.11	7.74 ± 0.02	0	0	100 100 100
	10.0	10.29 ± 0.13	7.70 ± 0.03	0	0	100 100 100
	16.0	16.53 ± 0.07	7.86 ± 0.03	0	0	60 70 60
1	7.0	7.18 ± 0.13 7.06 ± 0.19 6.98 ± 0.17	7.56 ± 0.01 7.57 ± 0.01 7.57 ± 0.01	28.65 ± 7.41 19.29 ± 4.91 24.45 ± 6.49	9.32 ± 2.40 6.14 ± 1.57 7.83 ± 1.99	100 100 100
	13.0	12.96 ± 0.07 12.86 ± 0.07 12.99 ± 0.10	7.60 ± 0.03 7.60 ± 0.02 7.61 ± 0.04	26.80 ± 6.95 29.05 ± 9.30 21.94 ± 4.07	7.16 ± 1.95 7.66 ± 2.50 5.71 ± 1.08	90 100 90
2	4.0	4.48 ± 0.12 4.76 ± 0.12 4.79 ± 0.16	7.68 ± 0.02 7.69 ± 0.02 7.69 ± 0.02	56.51 ± 11.27 59.67 ± 10.78 58.60 ± 8.11	16.18 ± 3.30 16.67 ± 3.22 16.41 ± 2.52	40 60 70
	10.0	10.05 ± 0.06 10.23 ± 0.05 10.01 ± 0.03	7.60 ± 0.02 7.62 ± 0.01 7.62 ± 0.02	56.51 ± 7.86 56.92 ± 9.52 39.56 ± 6.06	15.85 ± 2.30 15.47 ± 2.79 10.60 ± 1.72	70 60 100
	16.0	16.13 ± 0.12 16.13 ± 0.09 16.12 ± 0.07	7.84 ± 0.03 7.84 ± 0.03 7.85 ± 0.03	63.84 ± 8.24 55.93 ± 5.79 56.70 ± 4.90	10.21 ± 1.70 8.96 ± 1.20 8.96 ± 1.29	50 80 70
3	7.0	7.20 ± 0.23 7.00 ± 0.18 7.06 ± 0.12	7.55 ± 0.03 7.56 ± 0.02 7.58 ± 0.02	93.18 ± 13.22 100.13 ± 13.16 94.33 ± 14.74	30.58 ± 3.54 32.72 ± 4.32 29.45 ± 4.39	50 0 10
	13.0	12.94 ± 0.12 12.91 ± 0.04 13.02 ± 0.07	7.62 ± 0.03 7.64 ± 0.04 7.60 ± 0.02	101.15 ± 16.57 77.36 ± 2.86 87.00 ± 12.07	25.76 ± 4.71 19.18 ± 1.97 22.92 ± 3.27	0 0 0
4	10.0	10.64 ± 0.12 10.44 ± 0.16 10.61 ± 0.12	7.67 ± 0.04 7.68 ± 0.06 7.74 ± 0.04	135.22 ± 17.14 118.02 ± 14.49 126.69 ± 7.42	33.59 ± 3.87 32.59 ± 5.78 27.69 ± 3.00	0 0 0

* Observed temperature, pH and H₂S values are time-weighted mean±95% confidence intervals for observations at 0800 and 1400 hours each day of the test.

APPENDIX 11A CONTINUED
LAKE WHITEFISH SAC-FRY
96 HOUR BIOASSAY AT pH 8.0

Design H ₂ S Level	Design Temp °C	Observed Temp * °C	Observed pH	Total Sulphide ppB	Aqueous H ₂ S ppB	% Survival
0 (Control)	4.0	5.63 ± 0.14	8.08 ± 0.03	0	0	100 100 100
	10.0	10.43 ± 0.07	8.22 ± 0.02	0	0	100 100 100
	16.0	16.40 ± 0.05	8.29 ± 0.04	0	0	80 100 90
1	7.0	7.08 ± 0.12 7.04 ± 0.12 7.00 ± 0.10	8.13 ± 0.03 8.14 ± 0.02 8.13 ± 0.02	35.28 ± 6.33 25.94 ± 5.62 29.92 ± 8.04	4.05 ± 0.82 2.93 ± 0.64 3.42 ± 0.98	100 100 100
	13.0	12.94 ± 0.17 12.93 ± 0.14 12.97 ± 0.13	8.12 ± 0.05 8.13 ± 0.04 8.14 ± 0.04	31.14 ± 5.37 35.97 ± 4.15 29.39 ± 4.23	3.08 ± 0.70 3.10 ± 0.91 2.80 ± 0.48	80 100 100
2	4.0	4.61 ± 0.11 4.80 ± 0.10 4.87 ± 0.11	8.03 ± 0.03 8.03 ± 0.03 8.04 ± 0.03	67.58 ± 9.25 65.77 ± 12.80 72.35 ± 9.63	10.21 ± 1.57 9.92 ± 2.19 10.71 ± 1.72	100 70 70
	10.0	10.13 ± 0.08 10.28 ± 0.08 10.10 ± 0.14	8.16 ± 0.02 8.18 ± 0.02 8.18 ± 0.01	62.67 ± 9.94 56.16 ± 8.61 51.47 ± 7.21	6.19 ± 1.07 5.23 ± 0.88 4.79 ± 0.69	100 100 100
	16.0	15.96 ± 0.08 15.94 ± 0.07 15.96 ± 0.04	8.27 ± 0.04 8.27 ± 0.03 8.27 ± 0.03	69.68 ± 9.29 65.61 ± 9.20 59.39 ± 7.95	4.60 ± 0.80 4.35 ± 0.73 3.96 ± 0.66	100 90 100
3	7.0	7.02 ± 0.11 7.06 ± 0.20 7.09 ± 0.13	8.11 ± 0.03 8.12 ± 0.03 8.14 ± 0.02	123.74 ± 16.20 128.76 ± 24.93 100.82 ± 13.64	14.71 ± 2.15 15.13 ± 3.51 11.29 ± 1.58	0 20 70
	13.0	12.90 ± 0.15 12.90 ± 0.17 13.00 ± 0.17	8.14 ± 0.04 8.14 ± 0.04 8.13 ± 0.04	93.34 ± 11.91 94.39 ± 15.67 94.75 ± 12.76	8.86 ± 1.52 9.00 ± 1.80 9.06 ± 1.40	100 50 100
4	10.0	10.88 ± 0.32 10.39 ± 0.08 10.54 ± 0.10	8.19 ± 0.02 8.18 ± 0.02 8.20 ± 0.02	178.12 ± 28.94 180.10 ± 34.21 145.40 ± 5.96	16.11 ± 3.20 16.86 ± 2.94 12.99 ± 0.54	0 0 0

* Observed temperature, pH and H₂S values are time-weighted means
±95% confidence intervals for observations at 0800 and 1400 hours
each day of the test.

APPENDIX 11A CONTINUED
LAKE WHITEFISH SAC-FRY
96 HOUR BIOASSAY AT pH 8.5

Design H ₂ S Level	Design Temp °C	Observed Temp * °C	Observed pH	Total Sulphide ppB	Aqueous H: S ppB	% Survival
0 (Control)	4.0	5.79 ± 0.36	8.41 ± 0.04	< 1		100 100 100
	10.0	10.69 ± 0.57	8.47 ± 0.04	< 1		100 100 100
	16.0	16.49 ± 0.03	8.48 ± 0.05	< 1		60 40 50
1	7.0	7.15 ± 0.16 7.00 ± 0.20 7.01 ± 0.20	8.46 ± 0.05 8.46 ± 0.04 8.46 ± 0.05	57.44 ± 6.61 47.45 ± 5.33 53.20 ± 4.92	3.30 ± 0.51 2.72 ± 0.42 3.05 ± 0.43	100 100 100
	13.0	12.96 ± 0.10 12.80 ± 0.14 12.86 ± 0.12	8.50 ± 0.03 8.51 ± 0.04 8.52 ± 0.04	56.72 ± 4.34 51.41 ± 5.58 37.96 ± 11.95	2.46 ± 0.25 2.19 ± 0.35 1.61 ± 0.54	80 100 100
2	4.0	4.55 ± 0.25 4.83 ± 0.27 4.88 ± 0.27	8.44 ± 0.04 8.42 ± 0.04 8.41 ± 0.03	92.49 ± 5.02 104.41 ± 8.19 104.27 ± 5.32	6.08 ± 0.60 7.06 ± 0.76 7.23 ± 0.63	100 100 90
	10.0	10.28 ± 0.34 10.41 ± 0.30 10.27 ± 0.37	8.48 ± 0.04 8.48 ± 0.04 8.48 ± 0.03	96.38 ± 8.93 91.42 ± 7.06 85.95 ± 6.85	4.77 ± 0.53 4.50 ± 0.44 4.22 ± 0.47	100 90 90
	16.0	15.84 ± 0.15 15.86 ± 0.15 15.91 ± 0.17	8.49 ± 0.04 8.49 ± 0.04 8.49 ± 0.04	101.51 ± 7.41 103.05 ± 4.95 96.73 ± 8.37	4.17 ± 0.40 4.25 ± 0.42 3.99 ± 0.51	70 40 40
3	7.0	7.15 ± 0.18 7.15 ± 0.96 7.12 ± 0.20	8.45 ± 0.04 8.69 ± 0.04 8.46 ± 0.05	153.07 ± 12.03 163.94 ± 24.02 139.69 ± 17.22	9.00 ± 1.14 10.06 ± 1.75 8.09 ± 1.34	40 0 90
	13.0	12.86 ± 0.09 12.81 ± 0.09 12.91 ± 0.11	8.50 ± 0.04 8.49 ± 0.04 8.50 ± 0.04	157.44 ± 9.58 142.31 ± 7.86 135.53 ± 6.35	6.88 ± 0.74 6.29 ± 0.60 5.29 ± 0.64	10 30 70
4	10.0	10.84 ± 0.65 10.68 ± 0.71 10.74 ± 0.75	8.44 ± 0.06 8.44 ± 0.04 8.44 ± 0.05	229.41 ± 10.22 235.56 ± 16.59 197.63 ± 9.93	12.03 ± 1.58 12.55 ± 1.56 10.44 ± 1.25	0 0 0

* Observed temperature, pH and H₂S values are time-weighted means
±95% confidence intervals for observations at 0800 and 1400
hours each day of the test.

APPENDIX 11B - ANALYSIS OF LAKE WHITEFISH SAC-FRY BIOASSAY DATA.
TOTAL H₂S AT pH 7.5 FOR 96 HOURS

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	60700.6469	5	12140.13	34.56 *
LINEAR	56961.9170	2	28480.96	81.09 *
X ₁ LINEAR	5380.5620	1	5380.56	15.32 *
X ₂ LINEAR	51524.2603	1	51524.26	146.69 *
QUADRATIC	1526.3450	2	763.17	2.17
X ₁ QUAD.	519.6447	1	519.64	1.48
X ₂ QUAD.	974.4659	1	974.47	2.77
X ₁ * X ₂	309.1157	1	309.12	.98
RESIDUAL	32664.9736	93		
TOTAL	93355.6396	98		

REGRESSION:

$$Y = 101.575 + 1.12554X_1 - 0.684346X_2 - 0.168854X_1^2 - 0.0026480X_2^2 + 0.0189448X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean residual for model (99 Data Points) = 12.85%

* Indicates significance at 0.05 probability level.

APPENDIX 11C - ANALYSIS OF LAKE WHITEFISH SAC-FRY BIOASSAY DATA.
TOTAL H₂S AT PH 8.0 FOR 96 HOURS

ANALYSIS OF VARIANCE TABLE - TRANSFORMED DATA					APPROX. F	MIP
SOURCE	SS	D.F.	MS			
REGRESSION	25567.6548	5	5113.53		32.47	.41503-19 *
LINEAR	17746.3359	2	8873.17		56.07	.411031-12 *
X1 LINEAR	532.0362	1	532.04		3.48	.125698+00
X2 LINEAR	16213.2012	1	16213.20		106.12	.224555-11 *
QUADRATIC	5037.2452	2	2518.62		16.48	.125454-05 *
X1 QUAD.	1.8583	1	1.86		.01	.002313+00
X2 QUAD.	4891.7718	1	4891.77		21.95	.120521-05 *
X1 * X2	984.3815	1	984.38		6.44	.222186-01 *
TRANSFORM	1744.6313	1	1744.63		11.40	.246100-05 *
RESIDUAL	3972.4941	26	152.79			
TOTAL	29540.1489	32				
RELATIVE LIKELIHOOD OF NO TRANSFORMATION VS. ML ESTIMATES						.24610-02

REGRESSION:

$$Y^{0.6162} = 19.4184 - 0.291493X_1 - 0.0447306X_2 + 0.00381730X_1^2 - 0.000777483X_2^2 + 0.0074516X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean residual for model (33 Data Points) = 9.18%

* Indicates significance at 0.05 probability level.

APPENDIX 11D - ANALYSIS OF LAKE WHITEFISH SAC-FRY BIOASSAY DATA.
TOTAL H₂S AT PH 8.5 FOR 96 HOURS

ANALYSIS OF VARIANCE TABLE - TRANSFORMED DATA

SOURCE	SS	D.F.	MSG	APPROX. F	HLR
REGRESSION	21910.7668	5	4382.15	25.37	.202988-12 *
LINEAR	15454.9872	2	7727.49	44.73	.207372-10 *
X1 LINEAR	1210.1824	1	1210.18	7.01	.195179-01 *
X2 LINEAR	14728.9083	1	14728.91	85.26	.382350-10 *
QUADRATIC	5292.5397	2	2646.27	15.32	.263614-05 *
X1 QUAD.	279.4604	1	279.46	1.62	.369376+00
X2 QUAD.	5274.3089	1	5274.31	30.53	.271852-05 *
X1 * X2	55.3716	1	55.37	.32	.816959+00
TRANSFORM	3564.6201	1	3564.62	20.68	.650773-04 *
RESIDUAL	4491.5703	26	172.75		
TOTAL	26402.3372	32			
RELATIVE LIKELIHOOD OF NO TRANSFORMATION VS. HL ESTIMATES =					.65077-04

REGRESSION:

$$Y^{0.5004} = 9.38103 + 0.348999X_1 + 0.0113304X_2 - 0.0290756X_1^2 - 0.000292952X_2^2 + 0.000779324X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean residual for model (33 Data Points) = 11.12%

* Indicates significance at 0.05 probability level.

APPENDIX 11E - ANALYSIS OF LAKE WHITEFISH SAC-FRY BIOASSAY DATA.
AQUEOUS H₂S AT pH 7.5 FOR 96 HOURS

ANALYSIS OF VARIANCE TABLE - UNREPLICATED DATA

SOURCE	SS	D.F.	MS	F
REGRESSION	61057.4619	5	12219.49	15.22 *
LINEAR	54476.2329	2	27237.12	78.50 *
X ₁ LINEAR	10076.2900	1	10076.29	31.60 *
X ₂ LINEAR	50306.5100	1	50306.50	144.29 *
QUADRATIC	2301.6115	3	1150.31	3.32
X ₁ ² QUAD.	1160.9626	1	1160.96	3.17
X ₂ QUAD.	1026.0951	1	1026.09	2.96
X ₁ X ₂	594.0979	1	594.10	1.73
RESIDUAL	32268.1777	93	346.97	
TOTAL	93365.6396	98		

REGRESSION:

$$Y = 91.2157 + 3.15846X_1 - 0.783276X_2 - 0.255137X_1^2 - 0.0393667X_2^2 - 0.106334X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean residual for model (99 Data Points) = 12.48%

* Indicates significance at 0.05 probability level.

APPENDIX 11F - ANALYSIS OF LAKE WHITEFISH SAC-FRY BIOASSAY DATA.
AQUEOUS H₂S AT PH 8.0 FOR 96 HOURS

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	33021.0713	5	6604.21	32.07 *
LINEAR	19911.4558	2	9955.75	48.29 *
X1 LINEAR	267.7025	1	267.70	1.30
X2 LINEAR	18158.3516	1	18158.35	88.07 *
QUADRATIC	7613.4504	2	3806.73	18.46 *
X1 QUAD.	51.2902	1	51.29	.25
X2 QUAD.	7434.0387	1	7434.04	36.06 *
TOTAL	10.1100	1	10.11	.05
RESIDUAL	3566.8052	27	206.18	
TOTAL	36587.8765	32		

REGRESSION:

$$Y = 118.752 - 3.57804X_1 + 3.18068X_2 + 0.116682X_1^2 - 0.601255X_2^2 + 0.0409387X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean residual for model (33 Data Points) = 9.25%

* Indicates significance at 0.05 probability level.

APPENDIX 11G - ANALYSIS OF LAKE WHITEFISH SAC-FRY BIOASSAY DATA.
AQUEOUS H₂S AT PH 8.5 FOR 96 HOURS

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	35222.3613	5	7044.47	23.88 *
LINEAR	28073.9473	2	14037.97	47.58 *
X1 LINEAR	12076.1492	1	12076.15	40.93 *
X2 LINEAR	23698.3906	1	23698.39	80.29 *
QUADRATIC	5294.0204	2	2642.01	8.96 *
X1 QUAD.	1176.7276	1	1176.73	3.99
X2 QUAD.	4528.6378	1	4528.64	15.35 *
X1 * X2	491.2149	1	491.22	1.67
RESIDUAL	7966.5137	27	295.02	
TOTAL	43189.8750	32		

REGRESSION:

$$Y = 80.1833 + 7.39305X_1 + 4.86425X_2 - 0.535326X_1^2 - 0.827768X_2^2$$

$$Y = \% \text{ Survival}$$

$$X_1 = \text{Temperature } ^\circ\text{C}$$

$$X_2 = \text{Aqueous H}_2\text{S ppb}$$

Mean residual for model (33 Data Points) = 11.64%

* Indicates significance at 0.05 probability level.

APPENDIX 12A
ROUND WHITEFISH SAC-FRY
96 HOUR BIOASSAY AT pH 7.5

Design H ₂ S Level	Design Temp °C	Observed Temp ° °C	Observed pH	Total Sulphide ppb	Aqueous H ₂ S ppb	% Survival
0 (Control)	4.0	4.90±.12	7.72±.10	0	0	100
		4.90±.12	7.72±.10			100
		4.90±.12	7.72±.10			100
	10.0	9.81±.46	7.62±.08	0	0	100
		9.81±.46	7.62±.08			100
		9.81±.46	7.62±.08			100
	16.0	16.35±.18	7.73±.09	0	0	100
		16.35±.18	7.73±.09			100
		16.35±.18	7.73±.09			100
1	7.0	6.78±.14	7.59±.07	3.78± 6.43	1.35±2.51	100
		6.73±.23	7.59±.09	2.77± 3.44	0.97±1.36	100
		6.60±.29	7.59±.08	2.61± 2.40	0.84±0.83	100
	13.0	12.78±.42	7.62±.07	2.93± 1.23	0.76±0.34	100
		12.70±.46	7.60±.04	3.43± 1.59	0.91±0.41	100
		12.69±.43	7.58±.06	4.29± 1.95	1.16±0.45	20
2	4.0	4.50±.17	7.71±.08	10.71± 7.48	2.85±1.88	100
		4.49±.14	7.67±.10	8.37± 5.70	2.44±1.60	100
		4.46±.15	7.69±.11	6.86± 5.40	1.92±1.46	100
	10.0	9.79±.51	7.49±.06	11.31± 4.14	3.83±1.45	100
		9.89±.54	7.54±.05	13.57± 5.94	4.29±2.04	100
		9.75±.52	7.50±.04	9.44± 4.30	3.20±1.55	100
	16.0	16.10±.23	7.70±.09	13.00± 3.19	2.71±0.76	100
		16.08±.24	7.72±.10	10.01± 3.93	1.98±0.70	100
		16.07±.22	7.73±.10	10.68± 3.02	2.05±0.46	85.7
3	7.0	6.86±.24	7.58±.07	18.49±13.03	6.20±5.34	100
		6.71±.27	7.59±.07	21.57±16.74	7.21±6.87	100
		6.75±.15	7.60±.08	19.19±17.16	6.17±6.02	100
	13.0	12.65±.47	7.61±.07	13.09± 6.97	3.34±1.92	0
		12.71±.46	7.54±.05	16.75± 3.01	4.97±1.11	80
		12.82±.37	7.60±.07	16.44± 4.81	4.26±0.90	100
4	10.0	10.04±.44	7.57±.07	22.25± 9.45	6.71±2.97	100
		10.13±.52	7.57±.03	28.52± 8.72	8.36±2.29	100
		10.07±.53	7.64±.02	22.64± 6.03	5.97±1.36	100

APPENDIX 12A CONTINUED
ROUND WHITEFISH SAC-FRY
96 HOUR BIOASSAY AT pH 7.5 (TEST 2)

Design H ₂ S Level	Design Temp °C	Observed Temp * °C	Observed pH	Total Sulphide ppB	Aqueous H ₂ S ppB	% Survival
0 (Control)	4.0	4.99 ± 0.24	7.78 ± 0.03	0	0	100
		4.99 ± 0.24	7.78 ± 0.03			90
		4.99 ± 0.24	7.78 ± 0.03			100
	10.0	10.24 ± 0.12	7.71 ± 0.03	0	0	90
		10.24 ± 0.12	7.71 ± 0.03			100
		10.24 ± 0.12	7.71 ± 0.03			100
	16.0	16.43 ± 0.09	7.86 ± 0.03	0	0	100
		16.43 ± 0.09	7.86 ± 0.03			100
		16.43 ± 0.09	7.86 ± 0.03			100
1	7.0	6.79 ± 0.19	7.56 ± 0.03	7.35 ± 1.27	2.41 ± 0.44	100
		6.79 ± 0.26	7.58 ± 0.04	4.86 ± 1.09	1.54 ± 0.39	100
		6.58 ± 0.21	7.57 ± 0.03	5.70 ± 1.01	1.85 ± 0.36	100
	13.0	12.91 ± 0.08	7.59 ± 0.04	6.21 ± 1.17	1.68 ± 0.35	100
		12.83 ± 0.22	7.61 ± 0.04	11.27 ± 2.33	2.95 ± 0.70	100
		12.82 ± 0.18	7.62 ± 0.06	8.97 ± 1.53	2.31 ± 0.43	100
2	4.0	4.41 ± 0.25	7.69 ± 0.05	20.59 ± 1.26	5.81 ± 0.68	100
		4.52 ± 0.33	7.70 ± 0.03	13.15 ± 3.54	3.64 ± 0.89	100
		4.60 ± 0.26	7.73 ± 0.03	11.42 ± 3.25	3.04 ± 0.91	100
	10.0	10.25 ± 0.17	7.59 ± 0.03	15.67 ± 6.28	4.50 ± 1.81	100
		10.36 ± 0.20	7.66 ± 0.04	14.74 ± 4.06	3.79 ± 1.22	100
		10.20 ± 0.15	7.63 ± 0.04	17.53 ± 2.59	4.68 ± 0.53	100
	16.0	16.18 ± 0.12	7.80 ± 0.03	23.13 ± 3.85	3.95 ± 0.68	100
		16.25 ± 0.06	7.82 ± 0.03	19.25 ± 1.87	3.15 ± 0.36	100
		16.20 ± 0.08	7.83 ± 0.03	22.03 ± 3.66	3.58 ± 0.61	90
3	7.0	6.71 ± 0.20	7.55 ± 0.04	36.33 ± 5.57	12.16 ± 2.38	100
		6.69 ± 0.23	7.56 ± 0.04	34.09 ± 3.50	11.15 ± 1.58	100
		6.72 ± 0.16	7.61 ± 0.04	31.56 ± 1.73	9.61 ± 0.75	100
	13.0	12.81 ± 0.09	7.60 ± 0.04	34.89 ± 2.23	9.29 ± 1.06	100
		12.85 ± 0.10	7.61 ± 0.04	32.56 ± 2.08	8.46 ± 0.54	100
		12.88 ± 0.20	7.60 ± 0.04	32.94 ± 3.49	8.74 ± 1.22	100
4	10.0	11.23 ± 1.45	7.68 ± 0.05	41.27 ± 12.36	10.24 ± 3.18	100
		10.63 ± 0.17	7.68 ± 0.06	51.57 ± 18.65	12.93 ± 4.73	100
		10.62 ± 0.16	7.77 ± 0.05	45.37 ± 6.26	9.36 ± 1.47	90

* Observed temperature, pH and H₂S values are time-weighted means
±95% confidence intervals for observations at 0800 hours and
1400 hours each day of the test.

APPENDIX 12A CONTINUED
ROUND WHITEFISH SAC-FRY
96 HOUR BIOASSAY AT pH 7.5 (TEST 3)

Design H ₂ S Level	Design Temp °C	Observed Temp * °C	Observed pH	Total Sulphide ppB	Aqueous H ₂ S ppB	% Survival
0 (Control)	4.0	5.51 ± 0.11	7.74 ± 0.02	0	0	100
		5.51 ± 0.11	7.74 ± 0.02			100
		5.51 ± 0.11	7.74 ± 0.02			100
	10.0	10.29 ± 0.13	7.70 ± 0.03	0	0	100
		10.29 ± 0.13	7.70 ± 0.03			100
		10.29 ± 0.13	7.70 ± 0.03			100
	16.0	16.53 ± 0.07	7.86 ± 0.03	0	0	90
		16.53 ± 0.07	7.86 ± 0.03			80
		16.53 ± 0.07	7.86 ± 0.03			90
1	7.0	7.18 ± 0.13	7.56 ± 0.01	28.65 ± 7.41	9.32 ± 2.40	100
		7.06 ± 0.19	7.57 ± 0.01	19.29 ± 4.91	6.14 ± 1.57	90
		6.98 ± 0.17	7.57 ± 0.01	24.45 ± 6.49	7.83 ± 1.99	90
	13.0	12.96 ± 0.07	7.60 ± 0.03	26.80 ± 6.95	7.16 ± 1.95	100
		12.86 ± 0.07	7.60 ± 0.02	29.05 ± 9.30	7.66 ± 2.50	100
		12.99 ± 0.10	7.61 ± 0.04	21.94 ± 4.07	5.71 ± 1.08	90
2	4.0	4.48 ± 0.12	7.68 ± 0.02	56.57 ± 11.27	16.18 ± 3.30	100
		4.76 ± 0.12	7.69 ± 0.02	59.67 ± 10.78	16.67 ± 3.22	100
		4.79 ± 0.16	7.69 ± 0.02	58.60 ± 8.11	16.41 ± 2.52	80
	10.0	10.05 ± 0.06	7.60 ± 0.02	56.51 ± 7.86	15.85 ± 2.30	100
		10.20 ± 0	7.62 ± 0.07	65.87 ± 31.08	18.08 ± 10.46	0
		10.01 ± 0.03	7.62 ± 0.02	39.56 ± 6.06	10.60 ± 1.72	100
	16.0	16.13 ± 0.12	7.84 ± 0.03	63.84 ± 8.24	10.21 ± 1.70	90
		16.13 ± 0.09	7.84 ± 0.03	55.93 ± 5.79	8.96 ± 1.20	90
		16.12 ± 0.07	7.85 ± 0.03	56.70 ± 4.90	8.96 ± 1.29	40
3	7.0	7.20 ± 0.23	7.55 ± 0.03	93.18 ± 13.22	30.58 ± 3.54	80
		7.02 ± 0.14	7.55 ± 0.02	105.11 ± 16.12	34.34 ± 4.80	80
		7.06 ± 0.12	7.58 ± 0.02	94.33 ± 14.74	29.45 ± 4.39	100
	13.0	12.98 ± 0.21	7.63 ± 0.05	98.77 ± 31.55	24.73 ± 8.68	0
		12.92 ± 0.07	7.63 ± 0.03	82.39 ± 11.74	20.62 ± 3.07	0
		13.06 ± 0.11	7.61 ± 0.03	83.89 ± 4.09	21.80 ± 1.44	0
4	10.0	10.59 ± 0.14	7.67 ± 0.05	133.48 ± 26.26	32.84 ± 4.19	0
		10.45 ± 0.08	7.67 ± 0.03	130.39 ± 18.56	28.88 ± 5.75	0
		10.61 ± 0.12	7.74 ± 0.04	126.69 ± 7.42	27.69 ± 3.00	0

* Observed temperature, pH and H₂S values are time-weighted means ± 95% confidence intervals for observations at 0800 and 1400 Hours each day of the test

APPENDIX 12A CONTINUED
ROUND WHITEFISH SAC-FRY
96 HOUR BIOASSAY AT pH 8.0

Design H ₂ S Level	Design Temp °C	Observed Temp ° °C	Observed pH	Total Sulphide ppB	Aqueous H ₂ S ppB	% Survival
0 (Control)	4.0	5.63 ± 0.14	8.08 ± 0.03	0	0	100
		5.63 ± 0.14	8.08 ± 0.03			100
		5.63 ± 0.14	8.08 ± 0.03			100
	10.0	10.43 ± 0.07	8.22 ± 0.02	0	0	90
		10.43 ± 0.07	8.22 ± 0.02			60
		10.43 ± 0.07	8.22 ± 0.02			70
	16.0	16.40 ± 0.05	8.29 ± 0.04	0	0	60
		16.40 ± 0.05	8.29 ± 0.04			50
		16.40 ± 0.05	8.29 ± 0.04			20
1	7.0	7.08 ± 0.12	8.13 ± 0.03	35.28 ± 6.33	4.05 ± 0.82	60
		7.04 ± 0.12	8.14 ± 0.12	25.94 ± 5.62	2.93 ± 0.64	60
		7.00 ± 0.10	8.13 ± 0.02	29.92 ± 8.04	3.42 ± 0.98	80
	13.0	12.94 ± 0.17	8.12 ± 0.05	31.14 ± 5.37	3.08 ± 0.70	30
		12.93 ± 0.14	8.13 ± 0.04	35.97 ± 4.15	3.10 ± 0.91	20
		12.97 ± 0.13	8.14 ± 0.04	29.39 ± 4.23	2.80 ± 0.48	50
2	4.0	4.61 ± 0.11	8.03 ± 0.03	67.58 ± 9.25	10.21 ± 1.57	70
		4.80 ± 0.10	8.03 ± 0.03	65.77 ± 12.80	9.92 ± 2.19	100
		4.87 ± 0.11	8.04 ± 0.03	72.35 ± 9.63	10.71 ± 1.72	70
	10.0	10.13 ± 0.08	8.16 ± 0.02	62.67 ± 9.94	6.19 ± 1.07	70
		10.28 ± 0.08	8.18 ± 0.02	56.16 ± 8.61	5.23 ± 0.88	60
		10.10 ± 0.14	8.18 ± 0.01	51.47 ± 7.21	4.79 ± 0.69	40
	16.0	15.96 ± 0.08	8.27 ± 0.04	69.68 ± 9.29	4.60 ± 0.80	20
		15.94 ± 0.07	8.27 ± 0.03	65.61 ± 9.20	4.35 ± 0.73	0
		15.96 ± 0.04	8.27 ± 0.03	59.39 ± 7.95	3.96 ± 0.66	40
3	7.0	7.02 ± 0.11	8.11 ± 0.03	123.74 ± 16.20	14.71 ± 2.15	80
		7.06 ± 0.20	8.12 ± 0.03	128.76 ± 24.93	15.13 ± 3.51	50
		7.09 ± 0.13	8.14 ± 0.02	100.82 ± 13.64	11.29 ± 1.58	50
	13.0	12.90 ± 0.15	8.14 ± 0.04	93.34 ± 11.91	8.86 ± 1.52	10
		12.90 ± 0.17	8.14 ± 0.04	94.39 ± 15.67	9.00 ± 1.80	10
		13.00 ± 0.17	8.13 ± 0.04	94.75 ± 12.76	9.06 ± 1.40	50
4	10.0	10.88 ± 0.32	8.19 ± 0.02	178.12 ± 28.94	16.11 ± 3.20	0
		10.41 ± 0.07	8.18 ± 0.02	184.10 ± 24.64	17.06 ± 2.14	50
		11.15 ± 0.25	8.20 ± 0.02	149.69 ± 15.60	13.06 ± 1.24	60

* Observed temperature, pH and H₂S values are time-weighted means ± 95% confidence intervals for observations at 0800 and 1400 hours each day of the test.

APPENDIX 12A CONTINUED
ROUND WHITEFISH SAC-FRY
96 HOUR BIOASSAY AT pH 8.5

Design H ₂ S Level	Design Temp °C	Observed Temp * °C	Observed pH	Total Sulphide ppB	Aqueous H ₂ S ppB	% Survival
0 (Control)	4.0	5.79 ± 0.36	8.41 ± 0.04	< 1	0	71.4
		5.79 ± 0.36	8.41 ± 0.04	< 1	0	100.0
		5.79 ± 0.36	8.41 ± 0.04	< 1	0	100.0
	10.0	10.69 ± 0.57	8.47 ± 0.04	< 1	0	71.4
		10.69 ± 0.57	8.47 ± 0.04	< 1		57.1
		10.69 ± 0.57	8.47 ± 0.04	< 1		57.1
	16.0	16.49 ± 0.03	8.48 ± 0.05	< 1	0	20.0
		16.49 ± 0.03	8.48 ± 0.05	< 1		0
		16.48 ± 0.06	8.48 ± 0.09	< 1		0
1	7.0	7.15 ± 0.16	8.46 ± 0.05	57.44 ± 6.61	3.30 ± 0.51	100.0
		7.00 ± 0.20	8.46 ± 0.04	47.45 ± 5.33	2.72 ± 0.42	70.0
		7.01 ± 0.20	8.46 ± 0.05	53.20 ± 4.92	3.05 ± 0.43	100.0
	13.0	12.96 ± 0.10	8.50 ± 0.03	56.72 ± 4.34	2.46 ± 0.25	57.1
		12.80 ± 0.14	8.51 ± 0.04	51.41 ± 5.58	2.19 ± 0.35	71.4
		12.86 ± 0.12	8.52 ± 0.04	37.96 ± 11.95	1.61 ± 0.54	28.6
2	4.0	4.55 ± 0.25	8.44 ± 0.04	92.49 ± 5.02	6.08 ± 0.60	71.4
		4.83 ± 0.27	8.42 ± 0.04	104.41 ± 8.19	7.06 ± 0.76	71.4
		4.88 ± 0.27	8.41 ± 0.03	104.27 ± 5.32	7.23 ± 0.63	100.0
	10.0	10.28 ± 0.34	8.48 ± 0.04	96.38 ± 8.93	4.77 ± 0.53	57.1
		10.41 ± 0.30	8.48 ± 0.04	91.42 ± 7.06	4.50 ± 0.44	57.1
		10.27 ± 0.37	8.48 ± 0.03	85.95 ± 6.85	4.22 ± 0.47	14.3
	16.0	15.84 ± 0.15	8.49 ± 0.04	101.51 ± 7.41	4.17 ± 0.40	42.9
		15.86 ± 0.15	8.49 ± 0.04	103.05 ± 4.95	4.25 ± 0.42	57.1
		15.91 ± 0.17	8.49 ± 0.04	96.73 ± 8.37	3.99 ± 0.51	42.9
3	7.0	7.15 ± 0.18	8.45 ± 0.04	153.07 ± 12.03	9.00 ± 1.14	42.9
		7.15 ± 0.96	8.69 ± 0.04	163.94 ± 24.02	10.06 ± 1.75	28.6
		7.12 ± 0.20	8.46 ± 0.05	139.69 ± 17.22	8.09 ± 1.34	42.9
	13.0	12.85 ± 0.12	8.48 ± 0.04	155.03 ± 12.42	7.03 ± 0.98	0
		12.81 ± 0.09	8.49 ± 0.04	142.31 ± 7.86	6.29 ± 0.60	0
		12.91 ± 0.11	8.50 ± 0.04	135.53 ± 6.35	5.29 ± 0.64	28.6
4	10.0	10.50 ± 1.15	8.45 ± 0.12	235.44 ± 9.32	12.25 ± 3.17	0
		10.61 ± 0.71	8.44 ± 0.04	235.56 ± 16.59	12.55 ± 1.56	0
		10.74 ± 0.75	8.44 ± 0.05	197.63 ± 9.93	10.44 ± 1.25	0

* Observed temperature, pH and H₂S values are time-weighted means ± 95% confidence intervals for observations at 0800 and 1400 hours each day of the test.

APPENDIX 12B - ANALYSIS OF ROUND WHITEFISH SAC-FRY BIOASSAY DATA.
TOTAL H₂S AT PH 7.5 FOR 96 HOURS

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	46428.1255	5	9285.62	26.02 *
LINEAR	34400.7876	2	17200.39	48.21 *
X1 LINEAR	4658.9578	1	4658.96	13.06 *
X2 LINEAR	29789.7927	1	29789.79	83.49 *
QUADRATIC	6014.3176	2	3007.16	8.43 *
X1 QUAD.	207.6965	1	207.70	.58
X2 QUAD.	5836.8391	1	5836.84	16.36 *
X1 * X2	5003.0953	1	5003.10	14.02 *
RESIDUAL	33183.4824	93	356.81	
TOTAL	79611.6074	98		

REGRESSION:

$$Y = 105.802 - 2.19308X_1 + 0.858529X_2 + 0.106972X_1^2 - 0.00626376X_2^2 - 0.0758522X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean residual for model (99 Data Points) = 10.31%

* Indicates significance at 0.05 probability level.

APPENDIX 12C - ANALYSIS OF ROUND WHITEFISH SAC-FRY BIOASSAY DATA.
TOTAL H₂S AT PH 8.0 FOR 9 6 HOURS

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	17985.7065	5	3597.14	11.29 *
LINEAR	14648.0486	2	7324.02	22.99 *
X1 LINEAR	12348.7703	1	12348.77	38.76 *
X2 LINEAR	3545.4384	1	3545.44	11.13 *
QUADRATIC	823.5583	2	411.78	1.29
X1 QUAD.	59.4756	1	59.48	.19
X2 QUAD.	811.9074	1	811.91	2.55
X1 + X2	49.4475	1	49.45	.16
RESIDUAL	8602.1689	27	318.60	
TOTAL	26587.8755	32		

REGRESSION:

$$Y = 134.621 - 7.48792X_1 - 0.376025X_2 + 0.114885X_1^2 + 0.00163932X_2^2 - 0.00880785X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean residual for model (33 Data Points) = 14.03%

* Indicates significance at 0.05 probability level.

APPENDIX 12D - ANALYSIS OF ROUND WHITEFISH SAC-FRY BIOASSAY DATA.
TOTAL H₂S AT PH 8.5 FOR 96 HOURS

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	27140.8104	5	5428.16	16.21 *
LINEAR	15132.5419	2	7566.27	22.39 *
X1 LINEAR	4623.2506	1	4623.24	25.52 *
X2 LINEAR	7604.2908	1	7604.29	22.74 *
QUADRATIC	2430.4104	3	810.14	4.19
X1 QUAD.	213.7517	1	213.75	.63
X2 QUAD.	2294.3101	1	2294.31	6.76 *
X1 * X2	1959.0712	1	1959.07	5.80 *
RESIDUAL	9123.0327	27	337.89	
TOTAL	36507.4511	32		

REGRESSION:

$$Y = 164.203 - 12.6086X_1 - 0.329756X_2 + 0.218011X_1^2 - 0.00161371X_2^2 + 0.0398569X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Total H₂S ppb

Mean residual for Model (33 Data Points) = 13.54%

* Indicates significance at 0.05 probability level.

APPENDIX 12E - ANALYSIS OF ROUND WHITEFISH SAC-FRY BIOASSAY DATA.
 AQUEOUS H₂S AT PH 7.5 FOR 96 HOURS

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	45657.1709	5	9131.438	25.01 *
LINEAR	4592.7725	2	1296.399	32.85 *
X1 LINEAR	10801.7444	1	10801.74	29.58 *
X2 LINEAR	33800.7471	1	33800.25	92.57 *
QUADRATIC	2699.7045	2	1349.65	3.70
X1 QUAD.	30.0603	1	30.06	.08
X2 QUAD.	2620.4104	1	2620.41	7.18 *
X1 * X2	14012.4677	1	14013.47	38.38 *
RESIDUAL	33955.4345	93	365.11	
TOTAL	79611.6074	98		

REGRESSION:

$$Y = 89.2245 + 1.38536X_1 + 4.61850X_2 - 0.0421756X_1^2 - 0.064928X_2^2 - 0.520369X_1X_2$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean residual for model (99 Data Points) = 10.75%

* Indicates significance at 0.05 probability level.

APPENDIX 12F - ANALYSIS OF ROUND WHITEFISH SAC-FRY BIOASSAY DATA.
AQUEOUS H₂S AT pH 8.0 FOR 96 HOURS

ANALYSIS OF VARIANCE TABLE - TRANSFORMED DATA

SOURCE	SS	D.F.	MSS	APPROX. F	MLR
REGRESSION	14645.8964	5	2937.18	9.38	.172944-07*
LINEAR	11340.1855	2	5670.09	18.11	.256090-06*
X1 LINEAR	10073.4214	1	10073.42	32.17	.806770-06*
X2 LINEAR	3966.6856	1	3966.69	12.67	.917982-03*
QUADRATIC	105.7127	2	52.86	.17	.794155+00
X1 QUAD.	100.3407	1	100.34	.32	.803448+00
X2 QUAD.	2.1519	1	2.15	.01	.995287+00
X1 * X2	202.7191	1	202.72	.65	.644567+00
TRANSFORM	995.8909	1	995.89	1.06	.128314+00
RESIDUAL	7515.3018	24	313.14		
TOTAL	22201.1980	32			

RELATIVE LIKELIHOOD OF NO TRANSFORMATION VS. ML ESTIMATES = .12831+00

REGRESSION:

$$Y^{0.7796} = 41.4804 - 0.303799X_1^{1.667} - 2.58801X_2^{0.333} + 0.000914689X_2^{3.334} - 0.188107X_2^{0.666} - 0.0409439X_1^{1.667}X_2^{0.333}$$

Y = % Survival

X₁ = Temperature °C

X₂ = Aqueous H₂S ppb

Mean residual for model (33 Data Points) = 13.55%

* Indicates significance at 0.05 probability level.

APPENDIX 12G - ANALYSIS OF ROUND WHITEFISH SAC-FRY BIOASSAY DATA.
 AQUEOUS H₂S AT PH 8.5 FOR 96 HOURS

ANALYSIS OF VARIANCE TABLE - UNTRANSFORMED DATA

SOURCE	SS	D.F.	MSS	F
REGRESSION	26302.6411	5	5260.53	13.92 *
LINEAR	15163.0311	2	7581.52	20.07 *
X ₁ LINEAR	12752.9896	1	12752.99	33.75 *
X ₂ LINEAR	6719.0614	1	6719.06	17.78 *
QUADRATIC	2611.3686	2	1305.68	3.46
X ₁ QUAD.	412.8719	1	412.87	1.09
X ₂ QUAD.	1963.6915	1	1963.69	5.20 *
X ₁ * X ₂	540.6532	1	540.65	1.43
RESIDUAL	10201.2100	27	377.82	
TOTAL	36503.8511	32		

REGRESSION:

$$Y = 170.265 - 14.3621X_1 - 2.79775X_2 + 0.317223X_1^2 - 0.538257X_2^2$$

$$Y = \% \text{ Survival}$$

$$X_1 = \text{Temperature } ^\circ\text{C}$$

$$X_2 = \text{Aqueous H}_2\text{S ppb}$$

$$+ 0.412097X_1X_2$$

Mean residual for model (33 Data Points) = 14.30%

* Indicates significance at 0.05 probability level.

POOR

QUALITY

ORIGINAL

